EXHIBIT 230

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(54) **DOSAGES FOR TREATMENT WITH ANTI-ERBB2 ANTIBODIES**

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(51) **Int. Cl.**[7] **A61K 39/395**

(52) **U.S. Cl.** **424/138.1**; 424/131.1; 424/133.1; 424/134.1; 424/135.1; 424/136.1; 424/137.1; 424/139.1; 424/141.1; 424/142.1; 424/143.1; 424/144.1; 424/145.1; 424/146.1; 424/147.1; 424/150.1; 424/151.1; 424/152.1; 424/153.1; 424/154.1; 424/155.1; 424/156.1; 424/158.1; 424/172.1; 424/174.1

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(56) **References Cited**

U.S. PATENT DOCUMENTS

4,676,980	A		6/1987	Segal et al.
4,753,894	A		6/1988	Frankel et al.
4,816,567	A		3/1989	Cabilly et al.
4,935,341	A		6/1990	Bargmann et al.
4,943,533	A		7/1990	Mendelsohn et al.
4,968,603	A		11/1990	Slamon et al.
4,975,278	A		12/1990	Senter et al.
5,169,774	A		12/1992	Frankel et al.
5,183,884	A		2/1993	Kraus et al.
5,288,477	A		2/1994	Bacus
5,359,046	A		10/1994	Capon et al.
5,367,060	A		11/1994	Vandlen et al.
5,401,638	A		3/1995	Carney et al.
5,464,751	A		11/1995	Greene et al.
5,480,968	A		1/1996	Kraus et al.
5,578,482	A		11/1996	Lippman et al.
5,604,107	A		2/1997	Carney et al.
5,641,869	A		6/1997	Vandlen et al.
5,663,144	A		9/1997	Greene et al.
5,677,171	A		10/1997	Hudziak et al.
5,705,157	A		1/1998	Greene
5,720,937	A		2/1998	Hudziak et al.
5,720,954	A		2/1998	Hudziak et al.
5,725,856	A		3/1998	Hudziak et al.
5,726,023	A		3/1998	Cheever et al.
5,728,687	A		3/1998	Bissery
5,747,261	A		5/1998	King et al.
5,770,195	A		6/1998	Hudziak et al.
5,772,997	A		6/1998	Hudziak et al.
5,776,427	A		7/1998	Thorpe et al.
5,783,186	A		7/1998	Arakawa et al.
5,801,005	A		9/1998	Cheever et al.
5,821,337	A		10/1998	Carter et al.
5,824,311	A		10/1998	Greene et al.
5,834,229	A		11/1998	Vandlen et al.
5,837,243	A		11/1998	Deo et al.
5,837,523	A		11/1998	Greene et al.
5,840,525	A		11/1998	Vandlen et al.
5,846,538	A		12/1998	Cheever et al.
5,856,110	A		1/1999	Vandlen et al.
5,859,206	A		1/1999	Vandlen et al.
5,869,445	A		2/1999	Cheever et al.
5,876,712	A		3/1999	Cheever et al.
5,877,305	A		3/1999	Huston et al.
5,908,835	A		6/1999	Bissery
5,910,486	A		6/1999	Curiel et al.
5,922,845	A		7/1999	Deo et al.
5,939,531	A		8/1999	Wels et al.
5,968,511	A		10/1999	Akita et al.
5,977,322	A		11/1999	Marks et al.
5,985,553	A		11/1999	King et al.
6,015,567	A		1/2000	Hudziak et al.
6,028,059	A		2/2000	Curiel et al.
6,054,297	A		4/2000	Carter et al.
6,054,561	A		4/2000	Ring
6,096,873	A		8/2000	Schaefer et al.
6,123,939	A		9/2000	Shawver et al.
6,165,464	A		12/2000	Hudziak et al.
6,333,348	B1	*	12/2001	Vogel et al. 514/449

FOREIGN PATENT DOCUMENTS

EP	0003089	A1	7/1979
EP	0599274	A1	6/1994
EP	616812	A1	9/1994
EP	0711565	B1	8/1998
JP	3-240498		10/1991
JP	5-117165		5/1993
JP	5-170667		7/1993
JP	5-213775		8/1993
JP	5-317084		12/1993
JP	95006982	B2	1/1995
JP	7-59588		3/1995
JP	2761543	B2	6/1998
JP	2895105	B2	5/1999

(List continued on next page.)

OTHER PUBLICATIONS

Wantanabe et al., ASCO, Vol. 17, abstract 702, May 15–18, 1998 Annual Meeting.*

(List continued on next page.)

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(57) **ABSTRACT**

The present invention concerns the treatment of disorders characterized by the overexpression of ErbB2. More specifically, the invention concerns the treatment of human patients susceptible to or diagnosed with cancer overexpressing ErbB2 with anti-ErbB2 antibody.

33 Claims, 5 Drawing Sheets

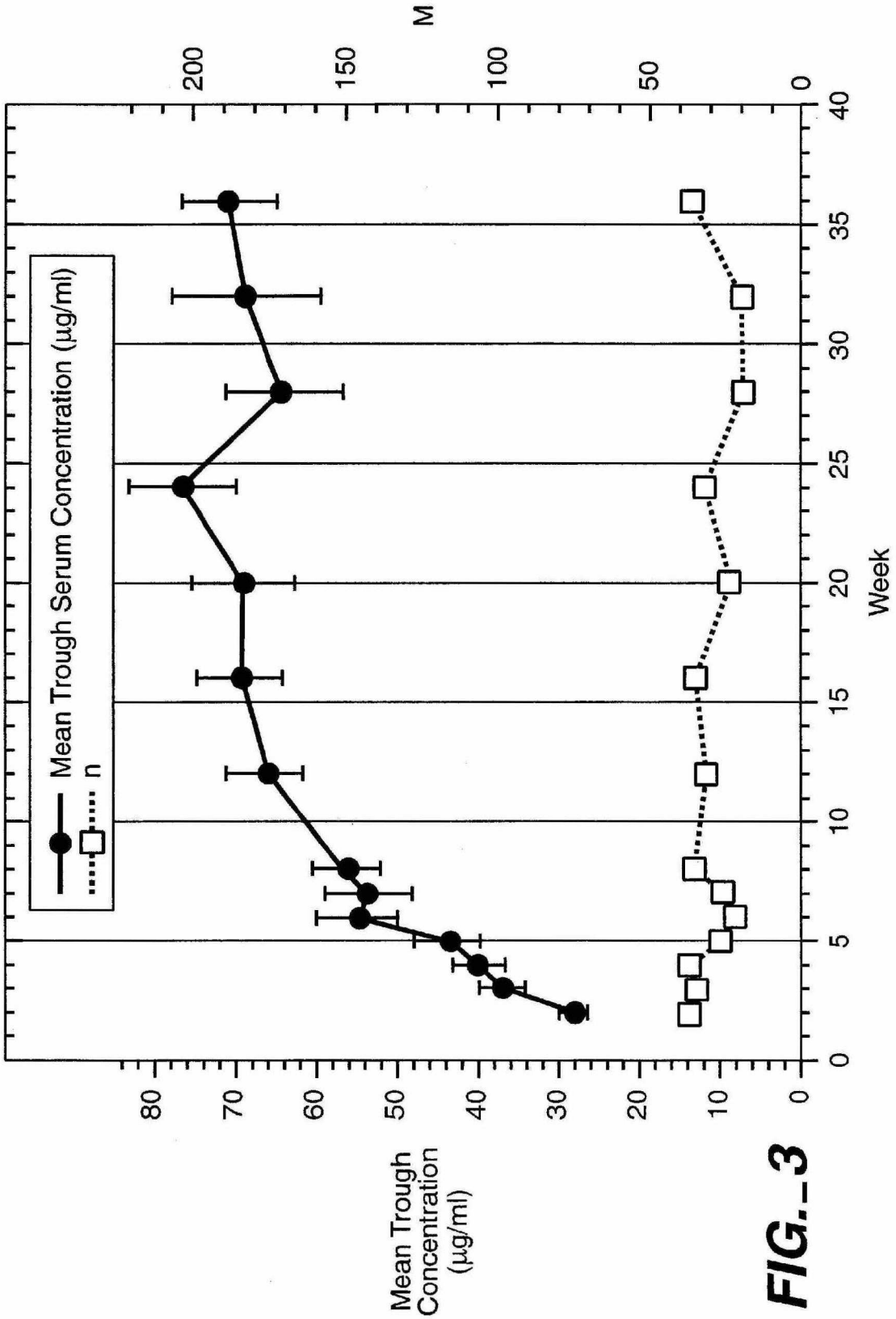
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FIG._3

DOSAGES FOR TREATMENT WITH ANTI-ERBB2 ANTIBODIES

RELATED APPLICATIONS

This application is a non-provisional application filed under 37 CFR 1.53(b)(1), claiming priority under 35 USC 119(e) to provisional application No. 60/151,018, filed Aug. 27, 1999 and No. 60/213,822, filed Jun. 23, 2000, the contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present invention concerns the treatment of disorders characterized by the overexpression of ErbB2 or disorders expressing epidermal growth factor receptor (EGFR), comprising administering to a human or animal presenting the disorders a therapeutically effective amount of an antibody that binds ErbB2. More specifically, the invention concerns the treatment of human patients susceptible to or diagnosed with cancer overexpressing ErbB2 or expressing EGFR, where the treatment is with an anti-ErbB2 antibody administered by front loading the dose of antibody during treatment by intravenous and/or subcutaneous administration. The invention optionally includes treatment of cancer in a human patient with a combination of an anti-ErbB2 antibody and a chemotherapeutic agent, such as, but not limited to, a taxoid. The taxoid may be, but is not limited to paclitaxel or docetaxel. The invention further includes treatment of cancer in a human patient with a combination of anti-ErbB2 antibody and a chemotherapeutic agent, such as, but not limited to, an anthracycline derivative. Optionally, treatment with a combination of anti-ErbB2 and an anthracycline derivative includes treatment with an effective amount of a cardioprotectant. The present invention further concerns infrequent dosing of anti-ErbB2 antibodies.

BACKGROUND OF THE INVENTION

Proto-oncogenes that encode growth factors and growth factor receptors have been identified to play important roles in the pathogenesis of various human malignancies, including breast cancer. It has been found that the human ErbB2 gene (erbB2, also known as her2, or c-erbB-2), which encodes a 185-kd transmembrane glycoprotein receptor ($p185^{HER2}$) related to the epidermal growth factor receptor (EGFR), is overexpressed in about 25% to 30% of human breast cancer (Slamon et al., *Science* 235:177–182 [1987]; Slamon et al., *Science* 244:707–712 [1989]).

Several lines of evidence support a direct role for ErbB2 in the pathogenesis and clinical aggressiveness of ErbB2-overexpressing tumors. The introduction of ErbB2 into non-neoplastic cells has been shown to cause their malignant transformation (Hudziak et al., *Proc. Natl. Acad. Sci. USA* 84:7159–7163 [1987]; DiFiore et al., *Science* 237:78–182 [1987]). Transgenic mice that express HER2 were found to develop mammary tumors (Guy et al., *Proc. Natl. Acad. Sci. USA* 89:10578–10582 [1992]).

Antibodies directed against human erbB2 protein products and proteins encoded by the rat equivalent of the erbB2 gene (neu) have been described. Drebin et al., *Cell* 41:695–706 (1985) refer to an IgG2a monoclonal antibody which is directed against the rat neu gene product. This antibody called 7.16.4 causes down-modulation of cell surface p185 expression on B104-1-1 cells (NIH-3T3 cells transfected with the neu proto-oncogene) a inhibits colony formation of these cells. In Drebin et al. *PNAS* (*USA*) 83:9129–9133 (1986), the 7.16.4 antibody was shown to

inhibit the tumorigenic growth of neu-transformed NIH-3T3 cells as well as rat neuroblastoma cells (from which the neu oncogene was initially isolated) implanted into nude mice. Drebin et al. in *Oncogene* 2:387–394 (1988) discuss the production of a panel of antibodies against the rat neu gene product. All of the antibodies were found to exert a cytostatic effect on the growth of neu-transformed cells suspended in soft agar. Antibodies of the IgM, IgG2a and IgG2b isotypes were able to mediate significant in vitro lysis of neu-transformed cells in the presence of complement, whereas none of the antibodies were able to mediate high levels of antibody-dependent cellular cytotoxicity (ADCC) of the neu-transformed cells. Drebin et al. *Oncogene* 2:273–277 (1988) report that mixtures of antibodies reactive with two distinct regions on the p185 molecule result in synergistic anti-tumor effects on neu-transformed NIH-3T3 cells implanted into nude mice. Biological effects of anti-neu antibodies are reviewed in Myers et al., *Meth. Enzym.* 198:277–290 (1991). See also WO94/22478 published Oct. 13, 1994.

Hudziak et al., *Mol. Cell. Biol.* 9(3):1165–1172 (1989) describe the generation of a panel of anti-ErbB2 antibodies which were characterized using the human breast tumor cell line SKBR3. Relative cell proliferation of the SKBR3 cells following exposure to the antibodies was determined by crystal violet staining of the monolayers after 72 hours. Using this assay, maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel,including 7C2 and 7F3, reduced cellular proliferation to a lesser extent in this assay. Hudziak et al. conclude that the effect of the 4D5 antibody on SKBR3 cells was cytostatic rather than cytotoxic, since SKBR3 cells resumed growth at a nearly normal rate following removal of the antibody from the medium. The antibody 4D5 was further found to sensitize p 185 -overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α. See also WO89/06692 published Jul. 27, 1989. The anti-ErbB2 antibodies discussed in Hudziak et al. are further characterized in Fendly et al. *Cancer Research* 50:1550–1558 (1990); Kotts et al. *In Vitro* 26(3):59A (1990); Sarup et al. *Growth Regulation* 1:72–82 (1991); Shepard et al. *J. Clin. Immunol.* 11(3):117–127 (1991); Kumar et al. *Mol. Cell. Biol.* 11(2):979–986 (1991); Lewis et al. *Cancer Immunol. Immunother.* 37:255–263 (1993); Pietras et al. *Oncogene* 9:1829–1838 (1994); Vitetta et al. *Cancer Research* 54:5301–5309 (1994); Sliwkowski et al. *J. Biol. Chem.* 269(20): 14661–14665 (1994); Scott et al. *J. Biol. Chem.* 266:14300–5 (1991); and D'souza et al. *Proc. Natl. Acad. Sci.*91:7202–7206 (1994).

Tagliabue et al. *Int. J. Cancer* 47:933–937 (1991) describe two antibodies which were selected for their reactivity on the lung adenocarcinoma cell line (Calu-3) which overexpresses ErbB2. One of the antibodies, called MGR3, was found to internalize, induce phosphorylation of ErbB2, and inhibit tumor cell growth in vitro.

McKenzie et al. *Oncogene* 4:543–548 (1989) generated a panel of anti-ErbB2 antibodies with varying epitope specificities, including the antibody designated TA1. This TA1 antibody was found to induce accelerated endocytosis of ErbB2 (see Maier et al. *Cancer Res.* 51:5361–5369 [1991]). Bacus et al. *Molecular Carcinogenesis* 3:350–362 (1990) reported that the TA1 antibody induced maturation of the breast cancer cell lines AU-565 (which overexpresses the erbB2 gene) and MCF-7 (which does not). Inhibition of growth and acquisition of a mature phenotype in these cells was found to be associated with reduced levels of ErbB2 receptor at the cell surface and transient increased levels in the cytoplasm.

administered is sufficient to maintain the target trough serum concentration such that the interval between administration cycles is at least one week. Preferably the trough serum concentration does not exceed 2500 μg/ml and does not fall below 0.01 μg/ml during treatment. The front loading drug treatment method of the invention has the advantage of increased efficacy by reaching a target serum drug concentration early in treatment. The subcutaneous delivery of maintenance doses according to the invention has the advantage of being convenient for the patient and health care professionals, reducing time and costs for drug treatment. Preferably, the initial dose (or the last dose within an initial dose series) is separated in time from the first subsequent dose by 4 weeks or less, preferably 3 weeks or less, more preferably 3 weeks or less, most preferably 1 week or less.

In an embodiment of the invention, the initial dose of anti-ErbB2 is 6 mg/kg, 8 mg/kg, or 12 mg/kg delivered by intravenous or subcutaneous administration, such as intravenous infusion or subcutaneous bolus injection. The subsequent maintenance doses are 2 mg/kg delivered once per week by intravenous infusion, intravenous bolus injection, subcutaneous infusion, or subcutaneous bolus injection. The choice of delivery method for the initial and maintenance doses is made according to the ability of the animal or human patient to tolerate introduction of the antibody into the body. Where the antibody is well-tolerated, the time of infusion may be reduced. The choice of delivery method as disclosed for this embodiment applies to all drug delivery regimens contemplated according to the invention.

In another embodiment, the invention includes an initial dose of 12 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 6 mg/kg once per 3 weeks.

In still another embodiment, the invention includes an initial dose of 8 mg/kg anti-ErbB2 antibody, followed by 6 mg/kg once per 3 weeks.

In yet another embodiment, the invention includes an initial dose of 8 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of8 mg/kg once per week or 8 mg/kg once every 2 to 3 weeks.

In another embodiment, the invention includes initial doses of at least 1 mg/kg, preferably 4 mg/kg, anti-ErbB2 antibody on each of days 1, 2 and 3, followed by subsequent maintenance doses of 6 mg/kg once per 3 weeks.

In another embodiment, the invention includes an initial dose of 4 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 2 mg/kg twice per week, wherein the maintenance doses are separated by 3 days.

In still another embodiment, the invention includes a cycle of dosing in which delivery of anti-ErbB2 antibody is 2–3 times per week for 3 weeks. In one embodiment of the invention, each dose is approximately 25 mg/kg or less for a human patient, preferably approximately 10 mg/kg or less. This 3 week cycle is preferably repeated as necessary to achieve suppression of disease symptoms.

In another embodiment, the invention includes a cycle of dosing in which delivery of anti-ErbB2 antibody is daily for 5 days. According to the invention, the cycle is preferably repeated as necessary to achieve suppression of disease symptoms.

The disorder preferably is a benign or malignant tumor characterized by the overexpression of the ErbB2 receptor, e.g. a cancer, such as, breast cancer, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer. The method of the invention may further comprise administration of a chemotherapeutic agent other than an anthracycline, e.g. doxorubicin or epirubicin. The chemotherapeutic agent preferably is a taxoid, such as TAXOL® (paclitaxel) or a TAXOL® derivative.

Preferred anti-ErbB2 antibodies bind the extracellular domain of the ErbB2 receptor, and preferably bind o the epitope 4D5 or 3H4 within the ErbB2 extracellular domain sequence. More preferably, the antibody is the antibody 4D5, most preferably in a humanized form. Other preferred ErbB2-binding antibodies include, but are not limited to, antibodies 7C2, 7F3, and 2C4, preferably in a humanized form.

The method of the present invention is particularly suitable for the treatment of breast or ovarian cancer, characterized by the overexpression of the ErbB2 receptor.

The present application also provides a method of therapy involving infrequent dosing of an anti-ErbB2 antibody. In particular, the invention provides a method for the treatment of cancer (e.g. cancer characterized by overexpression of the ErbB2 receptor) in a human patient comprising administering to the patient a first dose of an anti-ErbB2 antibody followed by at least one subsequent dose of the antibody, wherein the first dose and subsequent dose are separated from each other in time by at least about two weeks (e.g. from about two weeks to about two months), and optionally at least about three weeks (e.g. from about three weeks to about six weeks). For instance, the antibody may be administered about every three weeks, about two to about 20 times, e.g. about six times. The first dose and subsequent dose may each be from about 2 mg/kg to about 16 mg/kg; e.g. from about 4 mg/kg to about 12 mg/kg; and optionally from about 6 mg/kg to about 12 mg/kg. Generally, two or more subsequent doses (e.g. from about two to about ten subsequent doses) of the antibody are administered to the patient, and those subsequent doses are preferably separated from each other in time by at least about two weeks (e.g. from about two weeks to about two months), and optionally at least about three weeks (e.g. from about three weeks to about six weeks). The two or more subsequent doses may each be from about 2 mg/kg to about 16 mg/kg; or from about 4 mg/kg to about 12 mg/kg; or from about 6 mg/kg to about 12 mg/kg. The invention additionally provides an article of manufacture, comprising a container, a composition within the container comprising an anti-ErbB2 antibody, and a package insert containing instructions to administer the antibody according to such methods.

The presently described dosing protocols may be applied to other anti-ErbB antibodies such as anti-epidermal growth factor receptor (EGFR), anti-ErbB3 and anti-ErbB4 antibodies. Thus, the invention provides a method for the treatment of cancer in a human patient, comprising administering an effective amount of an anti-ErbB antibody to the human patient, the method comprising administering to the patient an initial dose of at least approximately 5 mg/kg of the anti-ErbB antibody; and administering to the patient a plurality of subsequent doses of the antibody in an amount that is approximately the same or less than the initial dose. Alternatively, or additionally, the invention pertains to a method for the treatment of cancer in a human patient comprising administering to the patient a first dose of an anti-ErbB antibody followed by at least one subsequent dose of the antibody, wherein the first dose and subsequent dose are separated from each other in time by at least about two

intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Intravenous or subcutaneous administration of the antibody is preferred.

The treatment of the present invention involves the administration of an anti-ErbB2 antibody to an animal or human patient, followed at intervals by subsequent doses of equal or smaller doses such that a target serum concentration is achieved and maintained during treatment. Preferably, maintenance doses are delivered by bolus delivery, preferably by subcutaneous bolus administration, making treatment convenient and cost-effective for the patient and health care professionals.

Where combined administration of a chemotherapeutic agent (other than an antracycline) is desired, the combined administration includes coadministration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. Preparation and dosing schedules for such chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service Ed., M. C. Perry, Williams & Wilkins, Baltimore, Md. (1992). The chemotherapeutic agent may precede, or follow administration of the antibody or may be given simultaneously therewith. The antibody may be combined with an anti-estrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP 616 812) in dosages known for such molecules.

It may be desirable to also administer antibodies against other tumor associated antigens, such as antibodies which bind to the EGFR, ErbB3, ErbB4, or vascular endothelial growth factor (VEGF). Alternatively, or additionally, two or more anti-ErbB2 antibodies may be co-administered to the patient. Sometimes, it may be beneficial to also administer one or more cytokines to the patient. The ErbB2 antibody may be co-administered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by the ErbB2 antibody. However, simultaneous administration, or administration of the ErbB2 antibody first is also contemplated. Suitable dosages for the growth inhibitory agent are those presently used and may be lowered due to the combined action (synergy) of the growth inhibitory agent and anti-ErbB2 antibody.

In addition to the above therapeutic regimens, the patient may be subjected to surgical removal of cancer cells and/or-radiation therapy.

For the prevention or treatment of disease, the appropriate dosage of anti-ErbB2 antibody will depend on the type of disease to be treated, as defined above, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Where the treatment involves a series of treatments, the initial dose or initial doses are followed at daily or weekly intervals by maintenance doses. Each maintenance dose provides the same or a smaller amount of antibody compared to the amount of antibody administered in the initial dose or doses.

Depending on the type and severity of the disease, about 1 μg/kg to 15 mg/kg (e.g. 0.1–20 mg/kg) of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. The progress of this therapy is easily monitored by conventional techniques and assays.

According to the invention, dosage regimens may include an initial dose of anti-ErbB2 of 6 mg/kg, 8 mg/kg, or 12 mg/kg delivered by intravenous or subcutaneous infusion, followed by subsequent weekly maintenance doses of 2 mg/kg by intravenous infusion, intravenous bolus injection, subcutaneous infusion, or subcutaneous bolus injection. Where the antibody is well-tolerated by the patient, the time of infusion may be reduced.

Alternatively, the invention includes an initial dose of 12 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 6 mg/kg once per 3 weeks.

Another dosage regimen involves an initial dose of 8 mg/kg anti-ErbB2 antibody, followed by 6 mg/kg once per 3 weeks.

Still another dosage regimen involves an initial dose of 8 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 8 mg/kg once per week or 8 mg/kg once every 2 to 3 weeks.

As an alternative regimen, initial doses of 4 mg/kg anti-ErbB2 antibody may be administered on each of days 1, 2 and 3, followed by subsequent maintenance doses of 6 mg/kg once per 3 weeks.

An additional regimen involves an initial dose of 4 mg/kg anti-ErbB2 antibody, followed by subsequent maintenance doses of 2 mg/kg twice per week, wherein the maintenance doses are separated by 3 days.

Alternatively, the invention may include a cycle of dosing in which delivery of anti-ErbB2 antibody is 2–3 times per week for 3 weeks. The 3 week cycle is preferably repeated as necessary to achieve suppression of disease symptoms.

The invention further includes a cyclic dosage regimen in which delivery of anti-ErbB2 antibody is daily for 5 days. According to the invention, the cycle is preferably repeated as necessary to achieve suppression of disease symptoms. Further information about suitable dosages is provided in the Examples below.

VI. Articles of Manufacture

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container, a label and a package insert. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-ErbB2 antibody. The label on, or associated with, the container indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes. In addition, the article of manufacture may comprise a package inserts with instruc-

intravenously, over a 90-minute period. Beginning on day 7, patients received weekly administration of 2 mg/kg antibody (i.v.) over a 90-minute period.

Chemotherapy

The patients received one of two chemotherapy regimens for a minimum of six cycles, provided their disease was not progressing: a) cyclophosphamide and doxorubicin or epirubicin (AC), if patients have not received anthracycline therapy in the adjuvant setting, or b) paclitaxel (T, TAXOL®), if patients have received any anthracycline therapy in the adjuvant setting. The initial dose of the HERCEPTIN® anti-ErbB2 antibody preceded the first cycle of either chemotherapy regimen by 24 hours. Subsequent doses of the antibody were given immediately before chemotherapy administration, if the initial dose of the antibody was well tolerated. If the first dose of the antibody was not well tolerated, subsequent infusions continued to precede chemotherapy administration by 24 hours. Patients were permitted to continue receiving chemotherapy beyond six cycles if, in the opinion of the treating physician, they were continuing to receive treatment benefit.

Cyclophosphamide (600 mg/m^2) was given either by iv push over a minimum period of 3 minutes or by infusion over a maximum period of 2 hours.

Doxorubicin (60 mg/m^2) or epirubicin (75 mg/m^2) were given either by slow iv push over a minimum period of 3–5 minutes or by infusion over a maximum period of 2 hours, according to institutional protocol.

Paciltaxel (TAXOL®) was given at a dose of 175 mg/m^2 over 3 hours by intravenous administration. All patients receiving paclitaxel were premedicated with dexamethasone (or its equivalent) 20 mg×2, administered orally 12 and 6 hours prior to paclitaxel; diphenhydramine (or its equivalent) 50 mg, iv, administered 30 minutes prior to paclitaxel, and dimetidine (or another H_2 blocker) 300 mg, iv, administered 30 minutes prior to paclitaxel.

Response Criteria

Progressive Disease

Objective evidence of an increase of 25% or more in any measurable lesion. Progressive disease also includes those instances when new lesions have appeared. For bone lesions, progression is defined as a 25% increase in objective measurement by plain film, CT, MRI; symptomatic new lesions not due to fracture; or requirement for palliative radiotherapy.

Complete Response

Disappearance of all radiographically and/or visually apparent tumor for a minimum of 4 weeks. Skin and chest wall complete responses had to be confirmed by biopsy.

Partial Response

A reduction of at least 50% in the sum of the products of the perpendicular diameters of all measurable lesions for a minimum period of 4 weeks. No new lesions may have appeared, nor may any lesions have progressed in size.

Minor Response

A reduction of 25% to 49% in the sum of the products of the perpendicular diameters of all measurable lesions. No new lesions may have appeared, nor may any lesions have progressed in size.

Stable Disease

No change of greater than 25% in the size of measurable lesions. No lesions may have appeared.

Time to disease progression (TTP) was calculated from the beginning of therapy to progression. Confidence limits for response rates were calculated using the exact method for a single proportion. (Fleiss, J L, *Statistical Methods for Rates and Proportions* (ed.2), New York, N.Y., Wiley, 1981, pp 13–17).

Results

At a median follow-up of 10.5 months, assessments of time to disease progression (TTP in months) and response rates (RR) showed a significant augmentation of the chemotherapeutic effect by HERCEPTIN® anti-ErbB2 antibody, without increase in overall severe adverse events (AE):

TABLE 1

HERCEPTIN ® Anti-ErbB2 Antibody Efficacy

	Enrolled	TTP (months)	RR (%)	AE (%)
CRx	234	5.5	36.2	66
CRx + 14	235	8.6*	62.00**	69
AC	145	6.5	42.1	71
AC + H	146	9.0	64.9	68
T	89	4.2	25.0	59
T + H	89	7.1	57.3	70

*$p < 0.001$ by log-rank test; ** $p < 0.01$ by X^2 test; CRx: chemotherapy; AC: anthracycline/cyclophosphamide treatment; H: HERCEPTIN ® anti-ErbB2 antibody; T: TAXOL ®

A syndrome of myocardial dysfunction similar to that observed with anthracyclines was reported more commonly with a combined treatment of AC+H (18% Grade ¾) than with AC alone (3%), T (0%), or T+H (2%).

These data indicate that the combination of anti-ErbB2 antibody treatment with chemotherapy markedly increases the clinical benefit, as assessed by response rates and the evaluation of disease progression. However, due to the increased cardiac side-effects of doxorubicin or epirubicin, the combined use of anthracyclines with anti-ErbB2 antibody therapy is contraindicated. The results, taking into account risk and benefit, favor treatment with HERCEPTIN® anti-ErbB2 antibody and paclitaxel (TAXOL®) where a combined treatment regimen is desired.

Example 2

Pharmacokinetic and Pharmacodynamic Properties of Anti-ErbB2 Antibody (HERCEPTIN®)

HERCEPTIN® anti-ErbB2 antibody was administered by intravenous, infusion to human patients selected according to the criteria provided in Example 1. An initial dose of 4 mg/kg HERCEPTIN® anti-ErbB2 antibody was delivered by intravenous infusion, followed by subsequent i.v. infusions of 2 mg/kg HERCEPTIN® anti-ErbB2 antibody weekly for several weeks. Two hundred thirteen patients began this treatment regimen and serum drug concentration was obtained beyond 8 weeks for fewer than 90 patients as selective discontinuation of patients with rapidly progressing disease occurred. Of the 213 patients who began treatment, serum trough concentration data were available for 80 patients at Week 12, for 77 patients at Week 16, for 44 patients at Week 20, for 51 patients at Week 24, for 25 patients at Week 28, for 23 patients at Week 32, and for 37 patients at Week 36.

HERCEPTIN® anti-ErbB2 Antibody Trough Serum Concentrations for Weeks 0–36

The HERCEPTIN® anti-ErbB2 antibody trough serum concentrations (μg/ml, mean±SE) from Week 2 through Week 36 are plotted in FIG. **3** (dark circles). The number of patients was fairly constant because data from patients discontinued from the program due to rapidly progressing disease were excluded from this analysis. Trough serum concentrations tended to increase through Week 12 and tended to plateau after that time.

HERCEPTIN® anti-ErbB2 Antibody Trough and Peak Serum Concentrations for Weeks 1–8

Some HERCEPTIN® anti-ErbB2 antibody serum concentration data were available for 212 of the original 213 patients. Trough and peak serum concentration data reflecting the first HERCEPTIN® anti-ErbB2 antibody infusion

were available for 195 of the 212 patients. For the seventh infusion, trough serum concentration data were available for 137/212 patients and peak serum concentration data were available for 114/212 patients. Table 2 presents a summary of statistics from trough and peak serum concentrations for the first 8 weeks of treatment. Peak samples were drawn shortly after the end of HERCEPTIN® anti-ErbB2 antibody administration; trough samples were drawn prior to the subsequent dose (i.e., 1 week later). Serum concentrations of HERCEPTIN® anti-ErbB2 antibody were determined as disclosed herein.

TABLE 2

HERCEPTIN ® Anti-ErbB2 Antibody Trough and Peak Serum Concentrations for the First 8 Weeks of Treatment (μg/ml)

	Dose Number	n	Mean	SD	Minimum	Maximum
Peak	1	195	100.3	35.2	30.7	274.6
Trough		195	25.0	12.7	0.16	60.7
Peak	2	190	74.3	31.3	20.8	307.9
Trough		167	30.4	16.0	0.2	74.4
Peak	3	167	75.3	26.8	16.1	194.8
Trough		179	33.7	17.9	0.2	98.2
Peak	4	175	80.2	26.9	22.2	167
Trough		132	38.6	20.1	0.2	89.4
Peak	5	128	85.9	29.2	27.8	185.8
Trough		141	42.1	24.8	0.2	148.7
Peak	6	137	87.2	32.2	28.9	218.1
Trough		115	43.2	24.0	0.2	109.9
Peak	7	114	89.7	32.5	16.3	187.8
Trough		137	48.8	24.9	0.2	105.2
Peak	8	133	95.6	35.9	11.4	295.6

The data in Table 2 suggest that there was an increase in trough serum concentration over time. Of the many patients studied, there were 18 patients for whom the trough concentrations did not exceed 20 μg/ml from Week 2 through Week 8. A HERCEPTIN® anti-ErbB2 antibody trough serum concentration of 20 μg/ml was nominally targeted for these studies based on prior pharmacologic studies in animals and exploratory analyses in clinical trials.

Patient response status was evaluated relative to serum concentration of HERCEPTIN® anti-ErbB2 antibody. For this purpose, mean serum concentration (an average of troughs and peaks) was calculated for various times and patient response status (where the patient response status was determined by an independent Response Evaluation Committee). The increase in serum concentration between Weeks 2 and 8 appeared to be greater in responders than in nonresponders, suggesting that there is a relationship between response status and HERCEPTIN® anti-ErbB2 antibody serum concentration. A statistical analysis (analysis of variance) of trough serum concentration values at Week 2 and an average of Weeks 7 and 8 in relation to response status indicated a highly significant relationship between response status and average trough of Weeks 7 and 8 ($p<0.001$). The results indicated that there was a significant difference between the trough serum concentration (average troughs of Weeks 7 and 8) in the responders and nonresponders: trough concentrations were 60±20 μg/ml in the responders versus 44±25 μg/ml in the nonresponders (mean±SD). HER2 overexpression level and type of metastatic sites were associated with significant differences in trough serum concentrations. At Week 2, patients with 2+ HER2 overexpression had significantly higher trough serum concentrations (n=40, mean=28.8 μg/ml, SD=10.4) compared with patients with 3+ HER2 overexpression (n=155, mean=24.1 μg/ml, SD=13.1). This difference in the average trough serum concentrations for Weeks 7 and 8 was no longer statistically significant. Further, at Week 2, patients with superficial disease had significantly higher trough serum concentrations (n=12, mean 34.1 μg/ml, SD=12.0) compared with patients with visceral disease (n=183, mean=24.4 μg/ml, SD=12.6). This difference in the average trough serum concentrations for Weeks 7 and 8 was significant. These data indicate that the rise in trough serum concentrations between Weeks 2 and 7/8 occurs for human patients with various disease profiles.

In a subsequent, similarly designed study, human breast cancer patients were treated with a loading dose of 8 mg/kg followed by maintenance doses of 4 mg/kg weekly. The results of this preliminary human study indicated that an 8mg/kg load:4 mg/kg weekly maintenance regimen was efficacious in reducing tumor volume in the patients.

The data disclosed in this Example indicate that front loading of antibody, such that a target serum concentration is reached more quickly, may be associated with improved outcomes.

Example 3

I.V. Bolus Delivery and Subcutaneous Infusion of HERCEPTIN® Anti-ErbB2 Antibody Effectively Decrease Tumor Volume in the Mouse

The efficacy of infusion or bolus delivery of humanized anti-ErbB2 antibody (HERCEPTIN®, see Example 1 for preparation), either by intravenous injection or subcutaneous injection, was examined. The purpose of the study was to ask whether subcutaneous delivery was feasible and whether the convenient subcutaneous bolus delivery was useful in treating metastatic breast cancer in animals inoculated with a cell line that overexpresses the HER2 gene. The results, detailed below, show that i.v. and s.c. infusion and bolus delivery are feasible treatment methodologies.

A study in a nude mouse xenograft model, which incorporates a human breast cancer cell line that naturally overexpresses the HER2 gene (BT-474MI, derived from BT-474 cells, ATCC Accession number HTB-20), comparing tumor volume as a function of i.v. bolus versus s.c. infusion was performed as follows. In the first study athymic nude nu nu 7–9 week old female mice were obtained from Taconic Inc (Germantown, N.Y.). To initiate tumor development, each mouse was inoculated subcutaneously with 3×10^6 BT474M 1 cells suspended in Matrigel™. When tumor nodules reached a volume of approximately 100 mm^3, animals were randomized to 4 treatment groups. The groups were treated according to Table 3.

TABLE 3

Animal Groups and Doses for Comparison of I.V. Bolus and S.C. Infusion

Group, Dose, Antibody	Target Serum Conc. μg/ml	Route of Administration	Loading Dose (mg/kg)	Maintenance Dose
1-Control, rhuMAb E25	20	IV LD and SC infusion	2.20	0.250 mg/ml (infusate)
2-Low Dose SC rhuMAb HER2	1	IV LD and SC infusion	0.313	0.050 mg/ml (infusate)
3-High Dose SC rhuMAb HER2	20	IV LD and SC infusion	6.25	1.00 mg/ml (infusate)
4-IV Multi-Dose rhuMAb HER2	20 (trough)	IV LD and MD	4.00	2 mg/kg/week (IV bolus)

Serum Conc. = concentration in serum.
LD = loading dose.
MD = maintenance dose.
Infusate concentration was calculated to achieve targeted serum concentration using Alzet ® osmotic minipumps (Alza Corp., Palo Alto, CA).

Animals were exposed to estrogen by subcutaneous sustained release estrogen pellet 9 days before the start of

TABLE 7

Efficacy of HERCEPTIN ® Anti-ErbB2 Antibody Measured as a Change in Tumor Volume Comparing Intravenous Bolus and Subcutaneous Bolus Delivery, Mean (SD)

Treatment Group (Delivery, MD)	Tumor Vol. Day 6, mm^3	Tumor Vol. Day 28 mm^3	Tumor Vol. Day 31, mm^3	Day 6–Day 31* Area Under Curve Tumor Vol., mm^3	Tumor Growth Rate on Log (TM + 1)
1-IV Control	321 (190)	1530 (1040)	1630 (1170)	13600 (7230)	0.0660 (0.0200)
2-IV Herceptin 1 mg/kg	297 (130)	175 (215)	151 (188)	4690 (1400)	–0.0505 (0.142)
3-IV Herceptin 2 mg/kg	269 (129)	75.7 (92.4)	73.6 (84.5)	3510 (1220)	–0.0608 (0.110)
4-IV Herceptin 4 mg/kg	272 (117)	25.3 (75.9)	25.8 (72.9)	2880 (1230)	–0.0810 (0.0859)
5-SC Herceptin 2 mg/kg	268 (117)	76.2 (98.8)	90.4 (105)	3230 (1440)	–0.0304 (0.104)

N = 10 for each data point.
TM = tumor measurement.
IV = intravenous.
SC = subcutaneous.
MD = maintenance dose.
Tumor Vol. = tumor volume, mm^3.
*Day 17 excluded due to measurement error.
Tumor growth rate calculated on Day 21–Day 31 Log(TM + 1). Area under the curve is the area beneath a plot of tumor volume versus time.

FIGS. **4**A and **4**B are graphical plots of changes in tumor volume over time, some of which data is found in Table 7. FIG. **4**A is a linear plot of tumor volume versus time. FIG. **4**B is a semilogarithmic plot of the same data, allowing the test points be viewed more clearly. The data in Table 7 and FIGS. **4**A and **4**B indicate that, although a dose-related response was not observed between HERCEPTIN-treated groups, dosing by subcutaneous bolus was as effective as intravenous bolus dosing and achieved similar trough serum concentrations.

Example 5

Regimens for Intravenous and Subcutaneous Delivery of Anti-ErbB2 Antibody

According to the invention, methods of anti-ErbB2 antibody (e.g., HERCEPTIN®) delivery comprise greater front loading of the drug to achieve a target serum concentration in approximately 4 weeks or less, preferably 3 weeks or less, more preferably 2 weeks or less, and most preferably 1 week or less, including one day or less. According to the invention, this initial dosing is followed by dosing that maintains the target serum concentration by subsequent doses of equal or smaller amount. An advantage of the methods of the invention is that the maintenance dosing may be less frequent and/or delivered by subcutaneous injection, making the treatment regimens of the invention convenient and cost-effective for the patient and medical professionals administering the antibody. In addition, a subcutaneous maintenance dose regimen may be interrupted by intravenous dosing (such as infusion) when the patient's chemotherapy requires delivery of other drugs by intravenous injection.

To test the following dosage regimens, human subjects are selected according to the criteria disclosed in Example 1, above. The number of initial doses is one or more doses sufficient to achieve an efficacious target serum concentration in approximately 4 weeks or less, preferably 3 weeks or less, more preferably 2 weeks or less, and most preferably 1 week or less, including 1 day or less. The number of maintenance doses may be one or more doses sufficient to achieve suppression of disease symptoms, such as a decrease in tumor volume. The maintenance doses are equal to or smaller than the initial dose or doses, consistent with an object of the invention of administering HERCEPTIN® anti-ErbB2 antibody by regimens providing greater front loading. The specific drug delivery regimens disclosed herein are representative of the invention and are not meant to be limiting.

In one trial, an initial dose of 6 mg/kg, 8 mg/kg, or 12 mg/kg of HERCEPTIN® anti-ErbB2 antibody is delivered to human patients by intravenous or subcutaneous injection. Initial doses (loading doses) are delivered by intravenous infusion or bolus injection or preferably subcutaneous bolus injection. Preferably a target trough serum concentration of HERCEPTIN® anti-ErbB2 antibody of approximately 10–20 μg/ml is achieved.(averaged for all patients in the treatment group) and maintained by subsequent doses of anti-ErbB2 antibody that are equal to or smaller than the initial dose. In one method, a target trough serum concentration is achieved and maintained by once-per-week deliveries of 2 mg/kg HERCEPTIN® anti-ErbB2 antibody by intravenous or subcutaneous injection for at least eight weeks. Alternatively, for this or any dosage regimen disclosed herein, subcutaneous continuous infusion by subcutaneous pump is used to delivery subsequent maintenance doses.

In another method, an initial (front loading) dose of 8 mg/kg HERCEPTIN® anti-ErbB2 antibody is delivered by intravenous injection (infusion or bolus injection) or by subcutaneous bolus injection. This is followed by intravenous bolus injections, intravenous infusion, subcutaneous infusion, or subcutaneous bolus injection of 6 mg/kg at 3-week intervals to maintain a trough serum concentration of approximately 10–20 μg/ml, averaged for an entire treatment group.

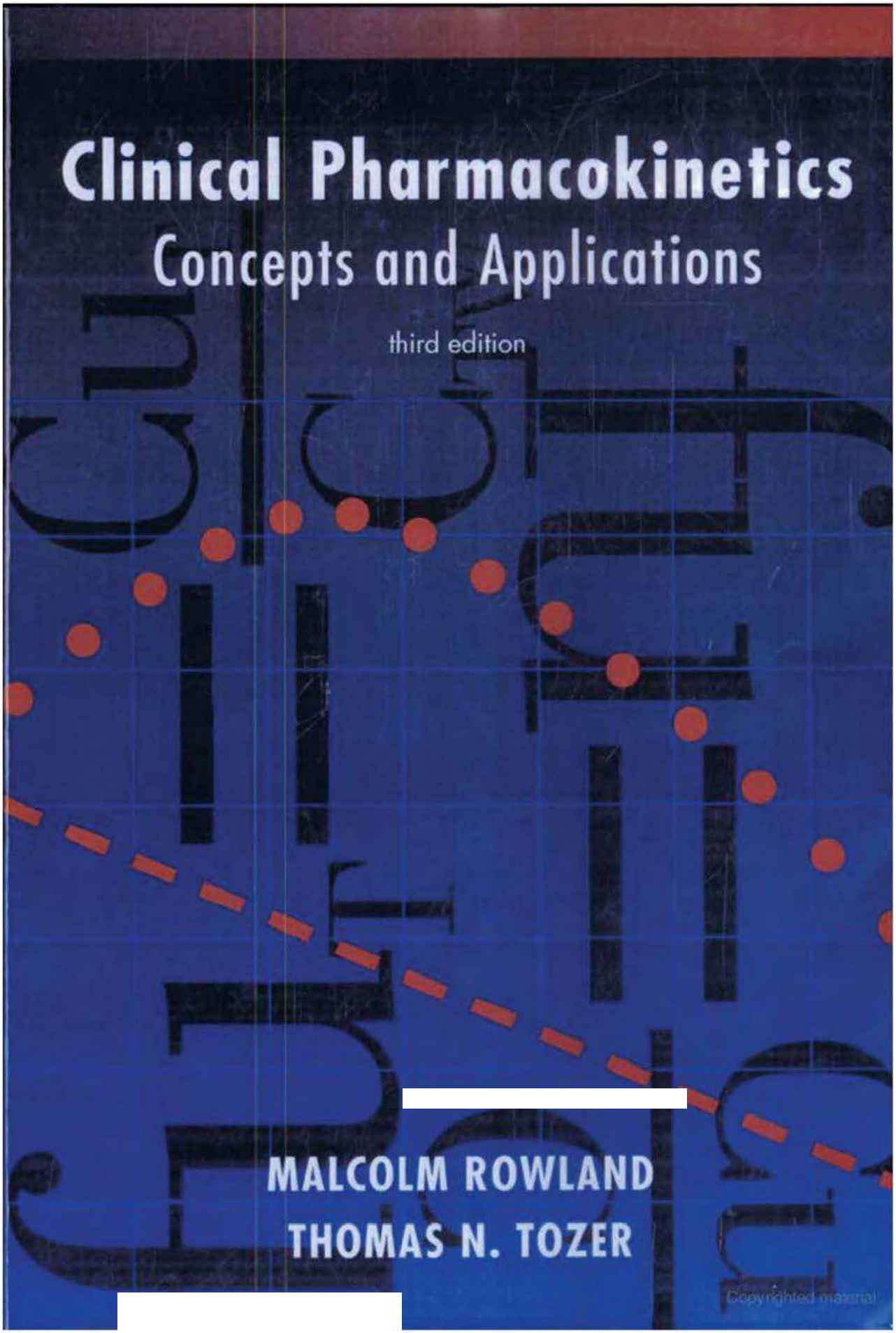
In another method, an initial (front loading) dose of 12 mg/kg HERCEPTIN® anti-ErbB2 antibody is delivered by intravenous injection (infusion or bolus injection) or by subcutaneous bolus injection. This is followed by intravenous bolus injections, intravenous infusion, subcutaneous infusion, or subcutaneous bolus injection of 6 mg/kg at 3-week intervals to maintain a trough serum concentration of approximately 10–20 μg/ml.

In yet another method, an initial (front loading) dose of 8 mg/kg HERCEPTIN® anti-ErbB2 antibody is delivered by intravenous infusion or bolus injection, or preferably by subcutaneous bolus injection or infusion.

This is followed by administration of 8 mg/kg per week or 8 mg/kg per 2–3 weeks to maintain a trough serum concentration of HERCEPTIN® anti-ErbB2 antibody of approximately 10–20 μg/ml. Maintenance doses are delivered by intravenous infusion or bolus injection, or preferably by subcutaneous infusion or bolus injection.

In another method, the front loading initial dose is a series of intravenous or subcutaneous injections, for example, one on each of days 1, 2, and 3 of at least 1 mg/kg for each injection (where the amount of anti-ErbB2 antibody delivered by the sum of initial injections is more than 4 mg/kg), followed by maintenance doses of 6 mg/kg once each 3 week interval to maintain a target trough serum concentration (for example, approximately 10–20 μg/ml) of HERCEPTIN® anti-ErbB2 antibody. The maintenance doses are delivered by intravenous infusion or bolus injection or by subcutaneous infusion or subcutaneous bolus injection.

EXHIBIT 231



Celltrion v. Genentech
IPR2017-01139 Genentech
Exhibit 2007

22

DOSE AND TIME DEPENDENCIES

OBJECTIVES

The reader will be able to:

1. List at least 10 sources of dose (or time) dependence in drug absorption, distribution, and elimination.
2. Recognize dose- (or time-) dependent kinetics from either plasma or urine data showing such behavior.
3. Graphically depict the kinetic behavior of a drug when the situation and the cause of a dose (or time) dependence are given.
4. On analyzing data in which a dose (or time) dependence occurs, identify which pharmacokinetic parameters are affected, and assign probable causes to the observation.
5. Demonstrate the kinetic consequences at steady state of a change in the rate of input, *Vm* or *Km*, of a drug showing saturable Michaelis-Menten metabolism.
6. Define saturable first-pass metabolism, describe how it can occur, and discuss its kinetic consequences.

An epileptic patient who has not responded to phenytoin after 2 weeks on 300 mg/day is observed to have a plasma concentration of 4 mg/L. Twenty days after the daily dose has been increased to 500 mg, the patient develops signs of toxicity, nystagmus, and ataxia; the plasma concentration of phenytoin is now 36 mg/L. Why should only a 67% increase in daily dose give rise to a ninefold increase in plasma concentration? The answer lies in the dose-dependent kinetic behavior of this drug.

Normally, plasma (or blood) concentration, unbound concentration, and amount of drug and its metabolites excreted in urine at any given time all increase in direct proportion to dose, when drug is administered in either a single dose or in multiple doses. Therefore, on correcting such observations for the dose administered, the values should superimpose at all times. This is referred to as the *principle of superposition*. When superposition occurs, the pharmacokinetics of a drug is said to be *dose-independent*, or *linear*.

There are many reasons why the principle of superposition may not hold. Among them are the administration of a drug by different routes, in different dosage forms, or by different methods (bolus or infusion). These are examples of dependencies on dosage form and route of administration. They are not the subject of this chapter. Other reasons for lack of superposition include changes in pharmacokinetic parameters themselves with size of dose administered or dosing rate, when all other factors are held constant. The pharmacokinetics of such drugs are said to be *dose-dependent*. When there is a lack of superposition on administering a drug on separate occasions or a lack of predictability following repeated or continuous dosing, based on single-dose data, the drug is said to show *time-dependent kinetics*. Such behaviors are sources of variability in drug response. Although

relatively uncommon, they occur frequently enough in drug therapy to warrant special consideration. In drug overdose, they are more the rule than the exception.

This chapter deals with identification and consequences of dose-dependent and time-dependent kinetics. The major intent is to establish a general awareness of this topic.

EVIDENCE

Five pharmacokinetic parameters (F, ka, V, CL_R, and CL_H) basically define and summarize the time-course of a drug in the body. Usually, none of these parameters systematically changes with dose in the same individual. But in *dose-dependent kinetics*, any one or a combination of these parameters appears to change with administration of different doses. The parameters change during continuous or repeated administration when kinetics show *time dependence*.

Both dose-dependent and time-dependent kinetic behaviors defy easy quantitative description and prediction. The first step in evaluating this behavior is to identify its occurrence. Subsequent steps involve determining the parameters affected and the likely mechanism(s) of the nonlinearity. There are many potential causes of dose and time dependencies. Table 22–1 lists examples of representative causes together with the pharmacokinetic parameters affected. Let us now consider examples of drugs for which there is evidence of nonlinear behavior in absorption or disposition. For many of these examples, therapeutic implications and means of accommodating or circumventing the problems are given.

ABSORPTION

Dose or time dependencies in drug absorption may be reflected by a change in either bioavailability or rate-time profile of absorption. These dependencies most often arise from three sources following oral administration. First are solubility and dissolution limitations in the release of drug from a dosage form in the gastrointestinal tract. Second is saturability

Table 22–1. Representative Causes of Dose- (or Time-) Dependent Kinetics and Selected Drug Examples

	EXAMPLE	PARAMETER AFFECTED[a]	
I. *Gastrointestinal Absorption*			
A. Saturable transport in gut wall	Amoxicillin	F	↓
B. Drug comparatively insoluble	Griseofulvin	F	↓
C. Saturable gut wall or hepatic metabolism on first pass	Nicardipine	F	↑
II. *Distribution*			
A. Saturable plasma protein binding	Naproxen	V, fu	↑
B. Saturable tissue binding		V, fu_T	↓, ↑
III. *Renal Excretion*			
A. Active secretion (saturable)	Penicillin G	Cl_R	↓
B. Active reabsorption (saturable)	Ascorbic acid	Cl_R	↑
C. Decrease in urine pH	Salicylic acid	Cl_R	↓
D. Saturable plasma protein binding	Disopyramide	Cl_R	↑
E. Nephrotoxicity[b]	Aminoglycosides	Cl_R	↓
F. Increase in urine flow[b]	Theophylline	Cl_R	↑
IV. *Hepatic Metabolism*			
A. Capacity-limited kinetics, cofactor limitation, etc.	Phenytoin	Cl_H	↓
B. Enzyme induction[b]	Carbamazepine	Cl_H	↑
C. Hepatotoxicity[b]	Acetaminophen	Cl_H	↓
D. Saturable plasma protein binding	Prednisolone	Cl_H	↑
E. Decreased hepatic blood flow	Propranolol	Cl_H	↓
F. Inhibition by metabolite[b]	Lidocaine	Cl_H	↓

[a]Direction of change: ↑ increase, ↓ decrease on increasing dose.
[b]Time-dependent as well as dose-dependent.

in a transport mechanism for passage across the gastrointestinal membranes. Last is saturability in metabolism during a drug's first pass through the gut wall and the liver.

Solubility

Dissolution can be the cause of dose dependency in bioavailability for drugs with low aqueous solubility, when given orally in relatively large doses. With a fixed transit time through the gastrointestinal tract, the amount of drug absorbed is unlikely to increase in proportion to the dose administered. An example is griseofulvin (Fig. 22–1). For this sparingly soluble drug (solubility is 10 mg/L), bioavailability decreases as the dose is increased from 250 to 500 mg.

Saturable Active Transport

For a few drugs, absorption from the gastrointestinal tract occurs by a capacity-limited transport mechanism. An example is that of amoxicillin, a polar β-lactam antibiotic. This drug is absorbed by a peptide transport mechanism in the small intestine. This conclusion is supported by the observation that bioavailability decreases with increased dose, but the peak time changes little (Fig. 22–2). The decrease in bioavailability is explained by the capacity-limited nature of the transport process. The lack of a major change in the peak time is a consequence of the limited region in the small intestine from which absorption can occur. The dose size does not influence the time between ingestion and movement past the site of transport.

Saturable First-Pass Metabolism

Nicardipine, a dihydropyridine calcium-channel blocker, exhibits dose dependence in its oral bioavailability (Table 22–2) because of saturability in its metabolism on first pass

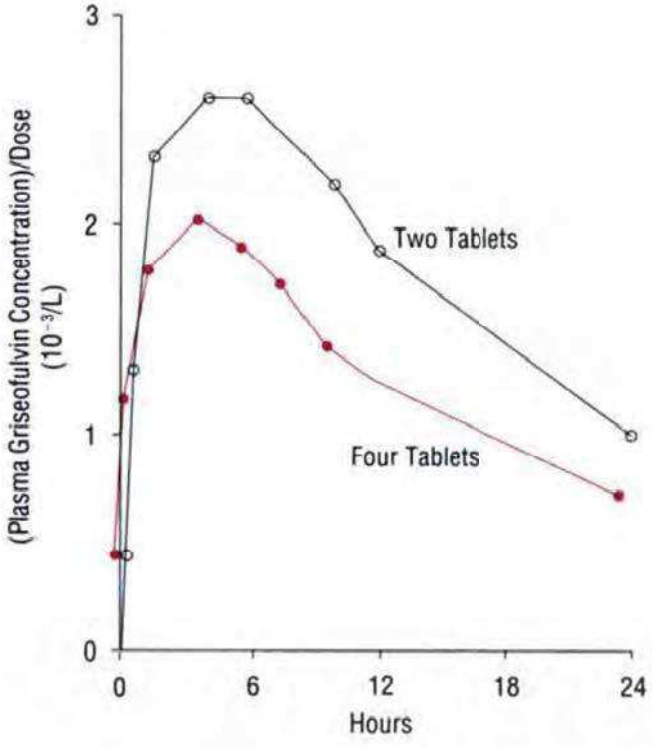


Fig. 22–1. Plasma concentration, normalized to dose, as a function of time following the oral administration of two tablets (upper curve ○) and four tablets (lower colored curve, ●) of ultramicronized griseofulvin (125 mg/tablet) (1 mg/L = 2.8 μM). (Adapted from data in Barrett, W.E., and Bianchine, J.R.: The bioavailability of ultramicronized griseofulvin (GRIS-PEG®) tablets in man. Curr. Ther. Res., *18*:501–509, 1975.)

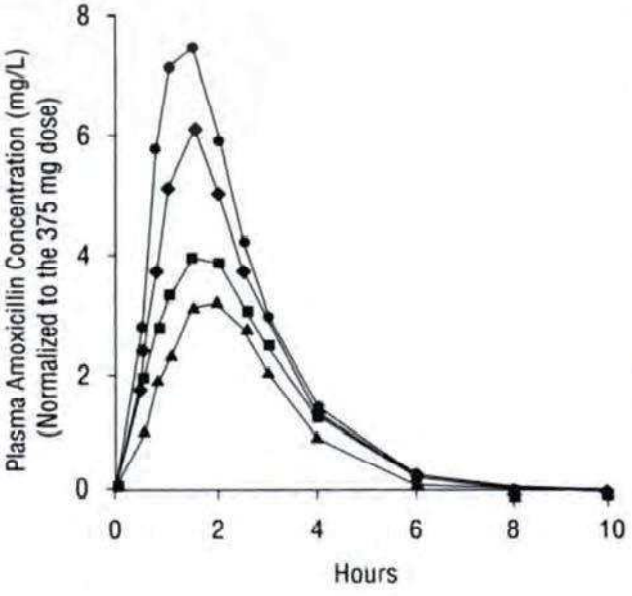


Fig. 22–2. Mean amoxicillin plasma concentrations after single oral doses of 375 (●), 750 (♦), 1500 (■), and 3000 (▲) mg. The concentrations are normalized to those expected for a 375-mg dose (C = C (observed) × 375/Dose(mg)). Note the decrease in the C_{max} and AUC values, and the similarity in t_{max} on increasing the dose. The observations are explained by oral bioavailability decreasing with dose with little or no change in peak time. (1 mg/L = 2.7 μM). (Data from Sjovall, J., Gunnar, A., and Westerlund, D.: Dose-dependent absorption of amoxicillin and bacampicillin. Clin. Pharmacol. Ther., 38:241–250, 1985. Interpretation from Reigner, B.G., Couet, W.R., Guedes, J.-P., Fourtillan, J.-B., and Tozer, T.N.: Saturable rate of cefatrizine absorption after oral administration to humans. J. Pharmacokinet. Biopharm., *18*:17–34, 1990.)

Table 22–2. Saturable First-Pass Metabolism of Nicardipine Observed at Steady State Following Oral Doses of 10 to 40 mg Every 8 hr[a]

DOSE (mg)	BIOAVAILABILITY (%)
10	19(4)[b]
20	22(5)
30	28(5)
40	38(6)

[a]Data from Wagner, J.G., Ling, T.L., Mroszczak, E.J., Freedman, D., Wu, A., Huang, B., Massey, I.J., and Roe, R.R.: Single intravenous dose and steady-state oral dose pharmacokinetics of nicardipine in healthy subjects. Biopharm. Drug Dispos., *8*:133–148, 1987.
[b]Mean and SE of data from 6 subjects.

through the liver. The data in the table were acquired during an 8-hr dosing interval at steady state (3 days into regimen). An i.v. radiolabeled tracer dose (0.885 mg) was given concurrently with the 30-mg dose to determine oral bioavailability.

Saturable first-pass metabolism occurs for a number of orally administered drugs that are highly extracted by the liver or intestinal tissues. Additional examples are listed in Table 22–3. For several of these drugs, dose dependence in oral bioavailability is observed without an apparent change in elimination half-life. This behavior can be understood by realizing that the concentration of drug reaching the liver during absorption can be much higher than that after absorption is over. Consider, for example, the following conditions: Distribution is instantaneous; all drug reaches the liver intact; input of drug from the gastrointestinal tract is first-order with a *ka* of 0.05 min-1 (14-min half-life); oral bioavailability at low doses is 0.1; volume of distribution is 250 L; total hepatic blood flow, Q_H, is 1.35 mL/min; and absorption is faster than elimination.

Table 22–3. Examples of Drugs Showing Saturable First-Pass Metabolism in the Liver or Gut Wall After Oral Administration of Therapeutic Doses

Alprenolol	Methoxysalen	Propranolol
5-Fluorouracil	Nicardipine	Salicylamide
Hydralazine	Propoxyphene	Verapamil

The initial rate of input into the portal vein, $ka \cdot \text{Dose}$, is also the initial rate of entry of drug into the liver, $Q_H \cdot C_{initial}$. Consequently, after a 100-mg dose,

$$C_{initial} = \frac{ka \cdot \text{Dose}}{Q_H} = 3.7 \text{ mg/L} \quad 1$$

The concentration in the blood entering the liver after absorption is finished would have a maximum value of

$$C_{max} = \frac{F \cdot \text{Dose}}{V} = 0.04 \text{ mg/L} \quad 2$$

The actual value of C_{max} should be less because some drug is eliminated during the absorption phase. As can be seen, the contribution of absorbed drug to the concentration entering the liver during first pass is much greater than that recycled from the rest of the body. Indeed, the ratio of $C_{initial}$ to C_{max} is

$$\frac{C_{initial}}{C_{max}} = \frac{ka \cdot V}{F \cdot Q_H} = 92 \quad 3$$

Thus, the larger the value of ka or V or the smaller the value of F at low (nonsaturating) doses, the greater is the ratio $C_{initial}/C_{max}$ and the more likely there is to be a separation in the degree of saturation of metabolism during absorption and elimination phases. All the drugs listed in Table 22–3 show saturable first-pass metabolism and have pharmacokinetic parameters that favor a high value of $C_{initial}/C_{max}$.

SATURABILITY OF PLASMA PROTEIN AND TISSUE BINDING

A limited number of binding sites exist on plasma proteins. Recall from Table 10–4 that the plasma concentration of albumin is usually 43 g/L or 600 μM (molecular weight = 67,000 g/mole). At one binding site per albumin molecule, there is then a limiting concentration of 600 μM for the bound drug. For α_1-acid glycoprotein, the limitation occurs at about 15 μM, a much lower concentration. The sites to which drugs bind in the tissues may be similarly limited. Consequently, the volume of distribution depends on drug concentration, a *concentration-dependent* behavior. Changes in binding tend to become appreciable when more than 20% of the available sites are occupied. This number, corresponding to 120 μM for one binding site on albumin, is arbitrary but useful for predicting the likelihood of concentration-dependent binding. For a drug with a molecular weight of 250, 120 μM corresponds to a concentration of 30 mg/L.

For drugs that show saturable binding to plasma proteins, the volume of distribution is expected to increase with plasma concentration, except when the volume of distribution is small (less than 0.2 L/kg, see Chap. 10, Distribution). Conversely, for drugs that show saturability in binding to tissues, the volume of distribution decreases as plasma concentration is increased. Because of the potential dependence on the fraction unbound in plasma and the dependence of half-life on both clearance and volume of distribution, dose dependence in distribution may be difficult to identify and quantify, unless plasma protein binding is measured. Consider the example of naproxen.

The *AUC* of naproxen following single doses fails to increase linearly with dose when doses above those maximally recommended (500 mg) are given (Fig. 22–3). Without any other information, this nonlinear observation might be explained by either a decrease in bioavailability or an increase in clearance, in that

$$AUC = \frac{F}{CL} \cdot \text{Dose} \qquad 4$$

The increase in clearance may be due to induction of metabolism or saturable binding to plasma proteins. As naproxen is a drug of low clearance (Dose/*AUC* calculated from data in Fig. 22–3A varies from 0.3 to 1.3 L/hr), the peak concentrations observed at doses of 1 to 4 g (Fig. 22–3B) provide information to distinguish between these possibilities. If one approximates a concentration of 110 mg/L when most of a 1000-mg dose is in the body, a value of *V/F* of approximately 9 L can be estimated. This small volume suggests strong binding to plasma proteins. The maximum concentrations obtained are in the region where nonlinear binding is expected for naproxen, a weak acid that binds to albumin. With a molecular weight of 230 g/mole, 100 and 200 mg/L correspond to concentrations of 430 and 870 μM, values approximating that (600 μM) of serum albumin. This is the condition in which *fu* is expected to increase with higher doses. Thus, with minimal information, a probable source of nonlinearity, saturable binding to plasma albumin, can be deduced.

The therapeutic consequence of decreased binding to plasma proteins at higher daily doses of a drug of low extraction ratio differs dramatically from that of drug-induced increased enzyme activity (autoinduction). When binding decreases (*fu* increases), the steady-state total plasma concentration is not increased much on doubling the rate of administration. The steady-state unbound concentration, however, doubles as a consequence of no change in unbound clearance. The intensities of toxic and therapeutic responses are expected to increase accordingly. In contrast, an increase in enzyme activity would affect both unbound and total concentrations proportionally. Thus, if autoinduction occurs, only a minor increase in response would be expected at higher rates of administration.

The expected change in the time-course of a drug in plasma when plasma protein binding exhibits nonlinear behavior is complex. Changes occur in both volume of distribution (Chap. 10) and clearance (Chap. 11). The magnitude of the changes depends on both the volume of distribution and the extraction ratio of the drug. Furthermore, because volume

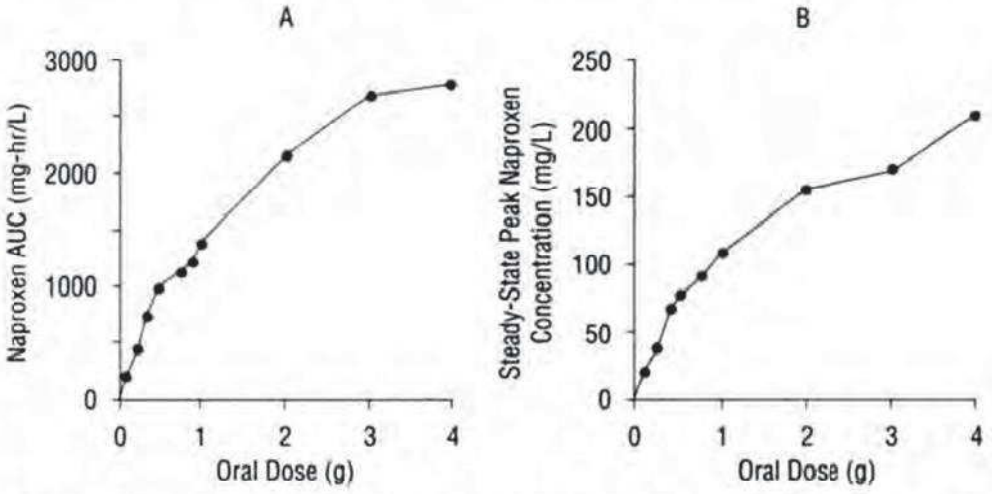


Fig. 22–3. The *AUC* of naproxen increases with the size of a single oral dose but not in direct proportion; the *AUC* appears to approach a limiting value (A). Nonlinearity is also observed in the peak concentration (B). These observations are consistent with either a decrease in *F* or an increase in *CL* with decreasing dose. As explained in the text, saturable binding to plasma albumin is most probably responsible (1 mg/L ≈ 4.3 μM). (Modified from Runkel, R., Chaplin, M.D., Sevelius, H., Ortega, E., and Segre, E.: Pharmacokinetics of naproxen overdoses. Clin. Pharmacol. Ther., *20*:269–277, 1976).

of distribution changes with amount in body, the decline of the plasma concentration does not reflect, in direct proportion, the disappearance of drug from the body. The slope of the semilogarithmic decline in the concentration-time curve is then not a good measure of the fractional rate of elimination. The qualitative effect of saturable binding to plasma proteins for a drug with a small volume of distribution is demonstrated by the decreasing slope of the unbound cefonicid concentration with time on a semilogarithmic plot (Fig. 22–4) after a single 30-mg/kg i.v. dose. The difference between the decline of the total and unbound concentrations (Fig. 22–4A) is explained by the decrease in the fraction unbound with time (Fig. 22–4B). The apparent one-compartmental nature of the total concentration decline is explained by virtually all drug in the body being bound to albumin.

The *ACE* inhibitor trandolaprilat, formed following oral administration of trandolapril, shows nonlinear plasma and tissue protein binding, as do other agents in this pharmacologic class. Evidence of nonlinearity in trandolaprilat kinetics is provided in Fig. 22–5. Both *AUC* (Fig. 22–5A) and plasma concentration (Fig. 22–5B), particularly during the terminal phase, fail to increase in direct proportion to the oral dose of trandolapril, over the eightfold range (0.5 to 4.0 mg) studied. Also, contrary to the expectation of linear kinetics, dosing daily, which is relatively frequent compared to the long terminal half-life, does not lead to extensive accumulation (Fig. 22–5C). Thus, when a 2-mg dose of trandolapril is administered orally for 10 days, the accumulation ratio of trandolaprilat ($AUC_{ss}/AUC_{(\tau,1)}$, Eq. 25, Chap. 7) is only 1.49. Direct evidence of concentration-dependent plasma protein binding of trandolaprilat is shown in Fig. 22–5D and Table 10–4. It should be noted that the range of concentrations, 0.5 to 5 μg/L, over which the fraction unbound changes threefold, covers most of the plasma trandiloprilat concentrations obtained following the oral doses of trandolapril (Fig. 22–5B, C).

The observations above can be rationalized as follows. The extremely low plasma concentration at which saturable binding occurs suggests a binding protein of much lower concentration than albumin or α_1-acid glycoprotein (see Table 10–4). The body of evidence

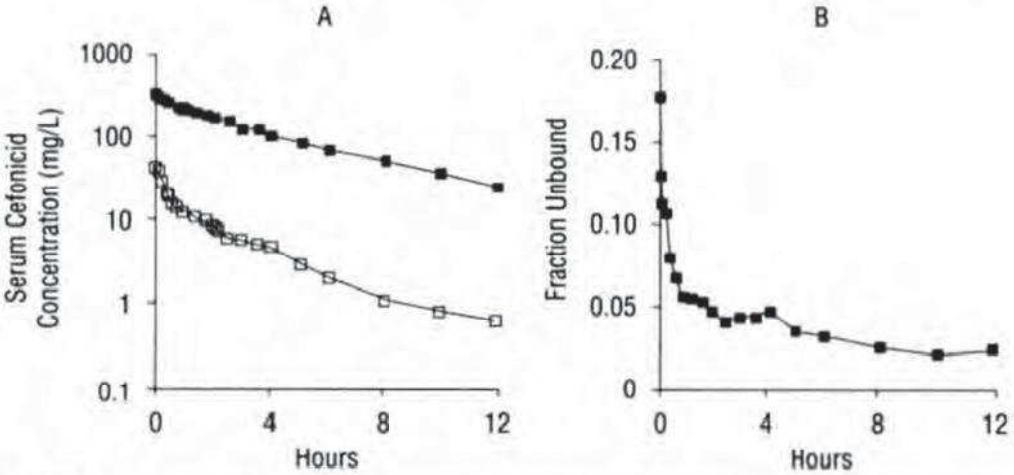


Fig. 22–4. *A*, Mean total (■) and unbound (□) plasma cefonicid concentrations with time in six volunteers after a single i.v. dose of 30 mg/kg given over 5 min. Note the much more rapid decline of the unbound concentration during the first 2 hr. *B*, The difference between the declines of the two curves in *A* is explained by a rapid decrease in the fraction unbound with time. As a consequence of the limited capacity of serum albumin to bind cefonicid, the high total concentrations at early times tend to approach the capacity for binding (1 mg/L = 1.84 μM). (Redrawn from Dudley, M.N., Shyu, W.-C., Nightingale, C.H., and Quintiliani, R.: Effect of saturable serum protein binding on the pharmacokinetics of unbound cefonicid in humans. Antimicrob. Agents Chemother.,

points to *ACE* itself being responsible. This high-affinity, low-capacity enzyme resides in both plasma and the endothelial linings of the vasculature, the latter site being interpreted as tissue binding when viewed from plasma data. Trandolaprilat, a relatively polar molecule, is restricted in its distribution mainly to extracellular spaces and is cleared systemically, mostly by renal excretion. Renal excretion is primarily via glomerular filtration, so that renal clearance shows concentration dependence associated with saturable protein binding. At high concentrations of trandolaprilat, which saturate *ACE*, renal clearance is high and elimination rapid. As the plasma concentration falls, the fraction bound to plasma and tissue *ACE* increases, thereby diminishing the unbound pool and lowering renal clearance. The net effect is a much slower elimination of material from the body. Thus, the biphasic decline of plasma trandolaprilat, seen in the semilogarithmic plots (Fig. 22–5B) is due to concentration-dependent protein binding and not distribution kinetics. Notice, that had only one dose of drug been administered, one could not have readily distinguished between concentration-dependent binding and distribution kinetics as the cause of the apparent biexponential decline of the plasma data. Finally, the lack of appreciable accumulation on multiple dosing arises because after each dose, most of the systemically available trandolaprilat is eliminated before reaching the terminal phase. Thus, nonlinear binding to the active site, *ACE*, appears to explain virtually all of trandolaprilat's odd kinetic behavior.

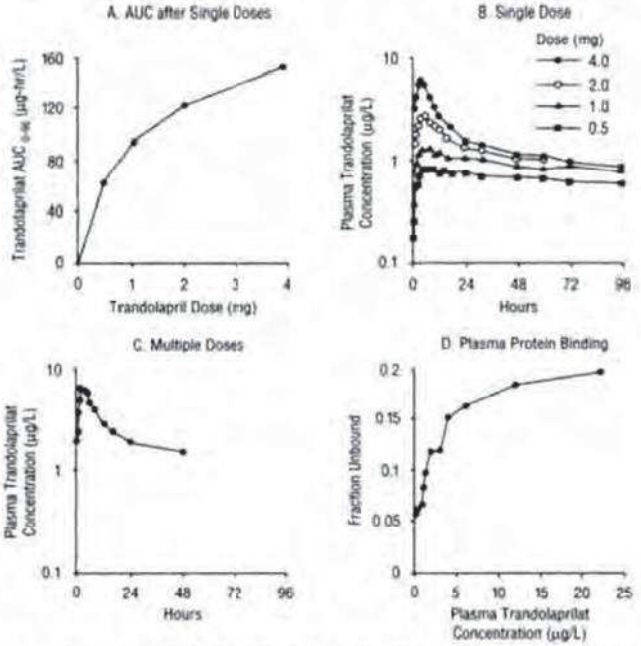


Fig. 22–5. Trandolaprilat, the active metabolite of trandolapril, exhibits nonlinear kinetic behavior. *A*, The *AUC* (0 to 96 hr) of the metabolite does not increase in direct proportion to the dose of trandolapril over the dose range of 0.5 to 4 mg. *B*, A semilogarithmic plot of mean plasma trandolaprilat concentrations after oral doses of 0.5, 1.0, 2.0, and 4.0 mg shows nonlinearity in that the curves are not equally spaced by a factor of 2 in the vertical direction for each of the successive doses. The lack of proportionality with dose is particularly evident at later times. *C*, Administration of 2.0 mg of trandolapril daily for 10 days fails to produce the degree of accumulation of trandolaprilat, as viewed from the concentration-time profile within a dosing interval at steady state (24 hr) that is predicted based on the long terminal decline in plasma concentration observed following a single 2.0-mg dose (see *B*). *D*, Binding of trandolaprilat to plasma proteins shows nonlinearity in that the fraction unbound increased with increasing concentration. (1 mg/L = 2.4 µM). (Data in *A* and *B* from Lenfant, B., Mouren, M., Bryce, T., De Lauture, D., and Strauch, G.: Trandolapril: Pharmacokinetics of single oral doses in male healthy volunteers, J. Cardiovasc. Pharmacol., in press, 1994; Data in *C* from Arnor, P., Wade, A., Engfelt, P., Mouren M., Stepniewski, J.P., Sultan, E., Bryce, T., and Lenfant, B: Pharmacokinetics and pharmacodynamics of trandolapril after repeated administration. J. Cardiovasc. Pharmacol., in press, 1994; Data in *D* from B. Lenfant, personal communication.)

CONCENTRATION-DEPENDENT RENAL EXCRETION

Renal clearance can vary with plasma concentration. Both filtration and reabsorption are usually passive processes, the rates of which are directly related to plasma concentration. In contrast, active secretion and active reabsorption are saturable processes with maximum capacities. This is shown in Fig. 22–6, for active secretion. The rate of tubular secretion increases in direct proportion to the plasma concentration until the transport approaches an upper limit often called the *TM* value. Consequently, clearance by secretion decreases as plasma concentration increases. This is observed for the antimicrobial agent dicloxacillin (Fig. 22–7). On increasing the dose from 1 to 2 g, the renal clearance, assessed by Ae_∞/AUC, is reduced. Extrarenal clearance is unaffected. With an *fu* of 0.04, the unbound renal clearance is about 2600 mL/min following the 1-g dose. This value greatly exceeds the usual glomerular filtration rate of 120 mL/min, indicating that this drug is extensively secreted into the tubular lumen (see Chap. 11, Elimination). At these doses, secretion shows concentration dependence and, as a consequence, the *AUC* increases disproportionately with dose. The body exposure to the drug and the half-life are disproportionately increased, considerations for the therapeutic use of large doses.

Secretion never occurs alone; filtration is always a component, and passive reabsorption may or may not be. Figure 22–6 also demonstrates how the rate of excretion of a drug that undergoes filtration and secretion, such as penicillin, always increases with plasma concentration. Even though the rate of secretion approaches an upper limit the rate of filtration continues to increase directly with unbound plasma concentration.

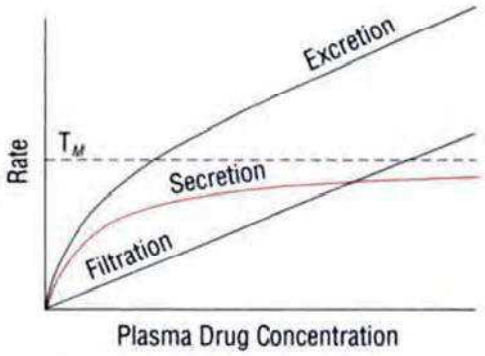


Fig. 22–6. The rate of renal secretion has a limiting value, the maximum transport rate (T_M), whereas the rate of filtration increases in direct proportion to the plasma concentration of a drug. Consequently, the rate of excretion of a drug that is both filtered and secreted, but not reabsorbed, increases with its plasma concentration. The increase, however, is not in direct proportion. Drug is either not bound in plasma or *fu* remains constant throughout the range of plasma concentrations.

Fig. 22–7. Renal clearance of dicloxacillin, as measured by Ae_∞/AUC, is decreased following a 2-g i.v. dose relative to that observed after a 1-g i.v. dose. The extrarenal clearance is not affected by dose. Saturable secretion of drug into the renal tubule explains the decrease in renal clearance. Mean ± SD (bars) (1 mg/L = 2.1 μM). (Data from Naufa, E.H., and Mattie, H.: Dicloxacillin and cloxacillin: Pharmacokinetics in healthy and hemodialysis subjects. Clin. Pharmacol. Ther., 20:98–108, 1976.)

Renal clearance is the rate of drug excretion divided by its plasma concentration. A drug that is only filtered, and is not bound in plasma, has the same renal clearance at all concentrations, as shown schematically in curve A of Fig. 22–8. Curve B depicts the events that occur for a drug that is actively secreted. In the region of plasma concentrations well below those required to approach saturation, renal clearance is highest and is relatively insensitive to changes in drug concentration. The therapeutic concentrations of most actively secreted drugs lie within this region. At higher plasma concentrations, renal clearance decreases; the lower limiting value is that contributed by both filtration and passive reabsorption.

The renal clearances of ascorbic acid, disopyramide, and the protein superoxide dismutase all increase with increasing concentrations, but for different reasons. Ascorbic acid (vitamin C) is normally conserved in the body by active reabsorption from the renal tubule. When the plasma concentration is excessive, the capacity of the reabsorption mechanism is exceeded, and the vitamin appears in large amounts in the urine (Fig. 22–9). The consequence of nonlinear excretion following oral administration of vitamin C is illustrated by the data in Table 22–4. Although statistically significant, note that the plasma concentration does not increase much even when megadoses are given. Another nonlinear mechanism also contributes to this observation. The bioavailability of the vitamin decreases with increasing dose (not shown) because it is absorbed in the intestine by a saturable process. The combined effect of saturable gastrointestinal absorption and renal tubular reabsorption is that only a relatively small change in the steady-state plasma concentration occurs, even when the daily oral dose is increased greatly.

Rather than saturable reabsorption, nonlinear binding to α_1-acid glycoprotein causes renal clearance (based on total concentration) of disopyramide to be greater at earlier times after a 1.5-mg/kg i.v. dose (Fig. 22–10A). Unbound renal clearance, on the other hand, shows no evidence of nonlinearity (Fig. 22–10B). The greater total clearance coincides with a higher total concentration and a lower binding ($fu \uparrow$) at earlier times.

The nonlinearity in renal clearance of the experimental protein drug superoxide dismutase is related to renal metabolism (Fig. 22–11). This protein, protective against cell injury that is due to oxygen-derived free radicals or superoxides, is filtered and then partially

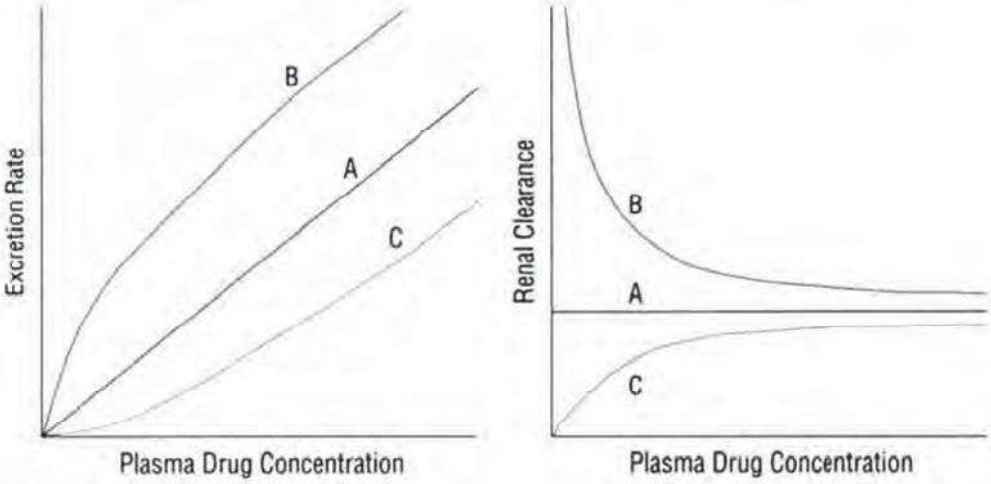


Fig. 22–8. Relationships between either rate of excretion (on left) or renal clearance (on right) and plasma concentration depend on whether the drug undergoes filtration only (curve A), filtration and secretion (curve B), or filtration and active reabsorption (curve C). The drug is either not bound to plasma proteins, or *fu* does not change in the concentration range shown.

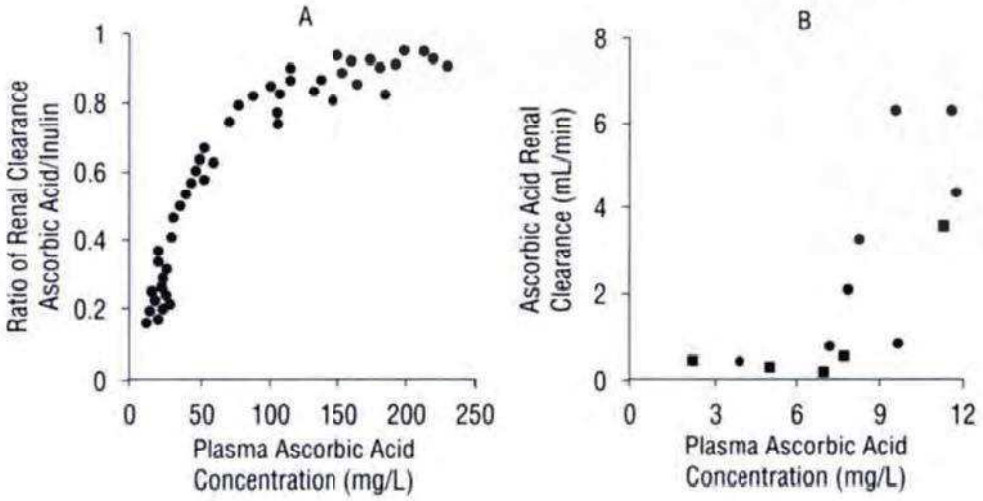


Fig. 22–9. *A*, The renal clearance of ascorbic acid relative to that of inulin increases dramatically at plasma concentrations well above the values of 4 to 15 mg/L typical for normal dietary conditions. The increase occurs at higher ascorbic acid plasma concentrations because the rate of filtration exceeds the capacity of the facilitated transport mechanism to reabsorb the vitamin from the tubular lumen. At high concentrations, the renal clearance of ascorbic acid approaches that of inulin, a compound that is only filtered. The high concentrations were produced following i.v. injections of 1500 to 6000 mg of ascorbic acid (1 mg/L = 5.7 μM). (Adapted from Ralli, E.P., Friedman, G.J., and Rubin, S.H.: The mechanism of the excretion of vitamin C by the human kidney. J. Clin. Invest., *17*:765–770, 1940.) *B*, In the plasma concentration range resulting from daily oral doses of 30 to 180 mg, renal clearance of ascorbic acid ranges from negligible to 4 to 8 mL/min (3 to 6% of the expected inulin clearance) in 22- to 45-year-old male subjects while on diets essentially free of vitamin C. Each symbol (●, ■) represents a group of subjects studied in one experiment with different intakes of ascorbic acid per day. (Adapted from Kallner, A., Hartmann, D., and Hornig, D.: Steady-state turnover and body pool of ascorbic acid in man. Am. J. Clin. Nutr., *32*:530–539, 1979.)

Table 22–4. Steady-State Trough Plasma Ascorbic Acid Concentrations in Healthy Adults Taking Various Doses of the Vitamin Twice Daily for 3 to 4 Weeks[a]

GROUP	PLASMA ASCORBIC ACID CONCENTRATION[b] (mg/L)
1. No supplementary dose, 6 subjects. Daily dietary intake of 50–75 mg expected	9 ± 0.6[c]
2. 1 to 3 g/day, 11 subjects	15.4 ± 1.6[d]
3. 8 to 12 g/day, 6 subjects	19.5 ± 2.0[d]

[a]Adapted from Yew, M.-L.S.: Megadose vitamin C supplementation and ascorbic acid and dehydroascorbic acid levels in plasma and lymphocytes. Nutr. Rep. Intern. *30*:597–601, 1984.
[b]Blood sampled in the morning before the next dose.
[c]Mean ± standard error of the mean.
[d]Significantly different from group 1.

metabolized in the renal tubular cells. At higher plasma concentrations, metabolism of the filtered drug becomes saturable with a resultant increase in renal clearance, an observation commonly reported for other proteins of comparable size. Note that the apparent upper limit of renal clearance (40 mL/min/70 kg) is less than the glomerular filtration rate, a probable consequence of filtration of this protein being incomplete because of its large molecular size (M.W. = 32,000 g/mole). Although no specific cut-off in molecular weight can be given, filtration of proteins above 30,000 g/mole falls off rapidly with increasing molecular size. Above 70,000 g/mole, only a small fraction is filtered except in glomerular disease (See Chap. 11, Elimination, p. 178).

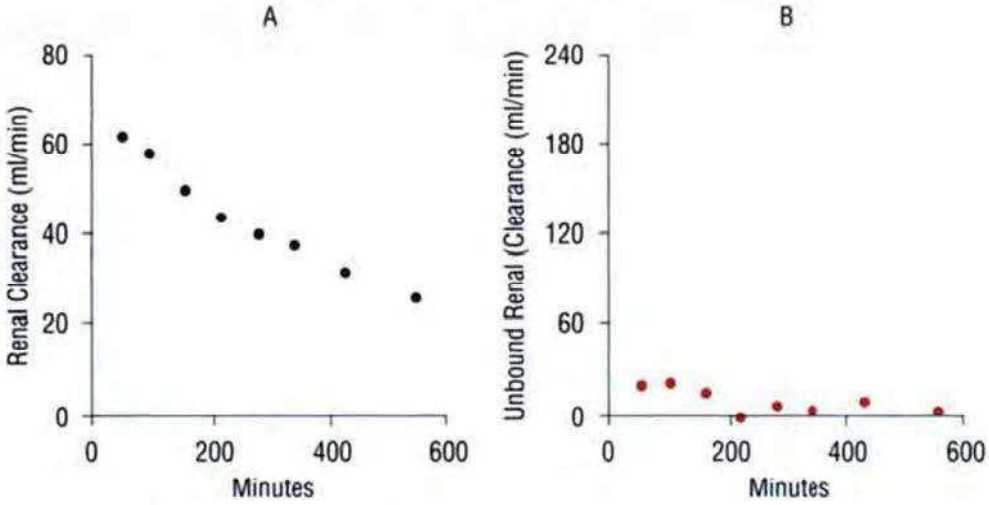


Fig. 22–10. *A*, The renal clearance of disopyramide in an individual subject shows changes with time after a single 1.5-mg/kg i.v. dose. *B*, Unbound renal clearance, on the other hand, does not appear to change over the corresponding time period. The initial plasma concentrations were in the range of 2 to 4 mg/L. These total concentrations are expected to produce nonlinear binding to α_1-acid glycoprotein, the protein to which this drug primarily binds in plasma (see Chap. 10, Distribution) (1 mg/L = 2.9 μM). (Modified from Giacomini, K.M., Swezey, S.E., Turner-Tamiyasu, K., and Blaschke, T.F.: The effect of saturable binding to plasma proteins on the pharmacokinetic properties of disopyramide. J. Pharmacokinet. Biopharm., *10*:1–14, 1982.)

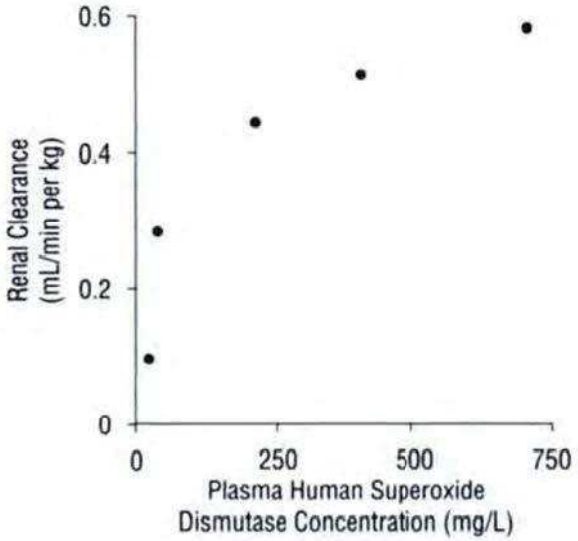


Fig. 22–11. Mean renal clearance of recombinant human superoxide dismutase increases at higher plasma concentrations after an i.v. dose of 45 mg/kg in eight subjects. The experimental drug is filtered in the glomerulus and partially metabolized by tubular cells. At higher plasma concentrations, the rate of filtration exceeds the ability of the tubular cells to absorb and metabolize the drug (1 mg/L = 0.031 μM). (Modified from Tsao, C., Greene, P., Odlind, B., and Brater, D.C.: Pharmacokinetics of recombinant human superoxide dismutase in healthy volunteers. Clin. Pharmacol. Ther., *50*:713–720, 1991.)

Renal clearance may also show concentration dependence when the drug (1) produces changes in pH and its tubular reabsorption is pH-dependent, e.g., salicylate; (2) is a diuretic and renal passive clearance is flow-dependent, e.g., theophylline; or (3) causes nephrotoxicity, e.g., an aminoglycoside. The mechanisms of the last two drugs are also time-dependent. Theophylline produces diuresis soon after its administration, but this effect, and consequently its renal clearance, decrease with time. The nephrotoxic effect of

aminoglycosides, on the other hand, develops with dose and duration of exposure to the drug.

CAPACITY-LIMITED METABOLISM

Perhaps the most dramatic dose-dependent kinetic mechanism is that of *capacity-limited metabolism*, a characteristic typical of enzymatic reactions. Recall from Chap. 11 (Eq. 11) that

$$\text{Rate of metabolism} = \frac{Vm \cdot Cu}{Km + Cu} \qquad 5$$

for a drug showing saturable Michaelis-Menten kinetics. Also, recall that in this case

$$\text{Unbound metabolic clearance} = \frac{Vm}{Km + Cu} \qquad 6$$

The social and therapeutic consequences of Michaelis-Menten kinetics are now explored with two examples, alcohol and phenytoin.

Alcohol

At doses usually consumed, the metabolism of alcohol is capacity-limited. Although metabolized by both alcohol dehydrogenase and cytochrome P450-2E1, the elimination kinetics of alcohol has been approximated by a Michaelis-Menten model of a single enzyme. This simplified kinetic model is subsequently presented.

The maximum rate of metabolism, *Vm*, and the Michaelis constant, *Km*, are approximately 10 g/hr and 100 mg/L, respectively. The pharmacologic effects of alcohol, which does not bind to plasma proteins, become apparent when the plasma concentration is about 200 mg/L, concentrations above 5000 mg/L are potentially lethal. Thus, the concentration range in which alcohol exerts its pharmacologic effects is well above its *Km*.

Table 22–5 shows the calculated rate of metabolism and clearance of alcohol as a function of the concentration at the metabolic site. Note that rate of metabolism of alcohol is essentially constant, zero-order, and close to *Vm* throughout the range of concentrations associated with activity. Accordingly, clearance decreases at high concentrations. At low concentrations, the intrinsic clearance (*Vm/Km*) approaches 100 L/hr or 1.6 L/min, a value

Table 22–5. Calculated Rate of Metabolism and Clearance of Alcohol as a Function of the Concentration at the Metabolic Site

CONCENTRATION AT SITE (mg/L)	RATE OF METABOLISM[a] (g/hr)	CLEARANCE[b] (L/hr)
7000	9.9	1.4
5000	9.8	2.0
3000	9.7	3.2
1000	9.1	9.1
500	8.3	17
200	6.7	33
100	5.0	50
50	3.3	67
10	0.91	91

[a]Rate of metabolism = $Vm \cdot Cu/(Km + Cu)$. Vm = 10 g/hr, Km = 100 mg/L.
[b]Clearance = $Vm/(Km + Cu)$.

in excess of hepatic blood flow. Thus, at very low concentrations, the extraction ratio is sufficiently high so that the rate of metabolism is partially limited by hepatic perfusion. Under these conditions oral bioavailability is expected to be reduced.

The consequences of zero-order elimination can be dramatic. The usual-size drink, 45 mL, of 40% v/v whiskey contains about 18 mL, or 14 g, of alcohol. Drinking this quantity of alcohol each hour exceeds the eliminating capacity of the body. Consequently, alcohol accumulates until ultimately either coma or death intervenes.

Alcohol distributes evenly throughout total body water; its volume of distribution is therefore 42 L. Accordingly, approximately 200 g of alcohol are needed in the body to achieve a concentration, about 5000 mg/L, that can produce coma or, occasionally, death. But, since the rate of ingestion, 14 g/hr, exceeds the rate of metabolism, 10 g/hr, by only 4 g/hr, this rate of drinking must be maintained for at least 2 days (a total of 48 drinks) to accumulate 200 g of alcohol. This degree of accrual can occur within 5 hr (20 drinks) when four drinks are consumed every hour, because this rate of ingestion, 56 g/hr, exceeds the maximum metabolic capacity by 46 g/hr.

If the rate of ingestion is reduced to one-half drink (or 7 g/hr), then, with respect to the effect of alcohol, one can drink with virtual impunity as now shown. By definition, of steady state, rate of elimination matches rate of administration (or input), R_o.

$$R_o = \frac{Vm \cdot Cu_{ss}}{Km + Cu_{ss}} \qquad 7$$

or on rearrangement

$$Cu_{ss} = \frac{Km \cdot R_o}{Vm - R_o} \qquad 8$$

Using the previously given values for *Km* and *Vm* and an R_o value of 7 g/hr, the plateau concentration of alcohol is 230 mg/L, which produced only a marginal effect.

Reflect on the calculations above. Chronically imbibing one-half a drink of whiskey per hour produces little or no effect, but taking one drink per hour becomes lethal. There can be no standard dosage regimen to maintain the effects of alcohol. Maintenance of effect requires titration of dosage to the effect itself.

The consequence of capacity-limited metabolism on the time-course of a drug in the body when input rate is changed is also demonstrated with alcohol. When alcohol is administered 10 min after ingesting water, light cream, or a glucose solution (80 g/240 mL), the plasma concentration-time profiles differ profoundly (Fig. 22–12). Compared to water, administration of light cream and 33% glucose, foods that delay gastric emptying, lower both *AUC* and peak concentration and increase time to reach the peak. These observations can be explained by the nearly zero-order metabolism of alcohol. To emphasize the point, assume that both elimination and input are strictly zero-order, as shown in Fig. 22–13. Decreasing the input rate, for a given total dose administered, lowers *AUC* and peak concentration as well as increases the peak time. Clearly, oral bioavailability in the presence of zero-order elimination and variable input rates cannot be assessed by conventional area ratio methods.

Phenytoin

Therapeutic problems encountered with capacity-limited metabolism are classically exemplified by phenytoin. Typical *Vm* and *Km* values of this drug are 500 mg/day and 0.4 mg/L, although the values vary widely. The value of *Km* is usually expressed in terms of

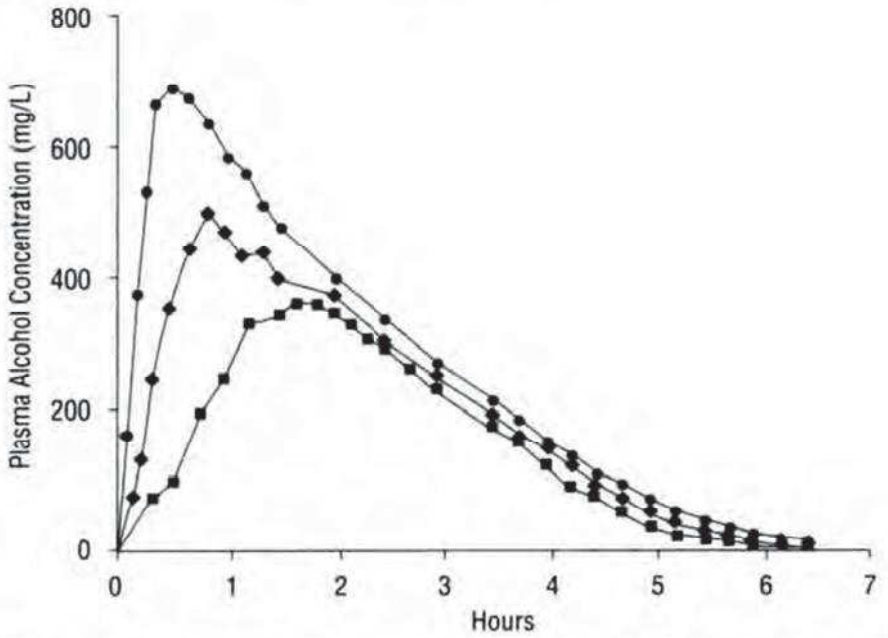
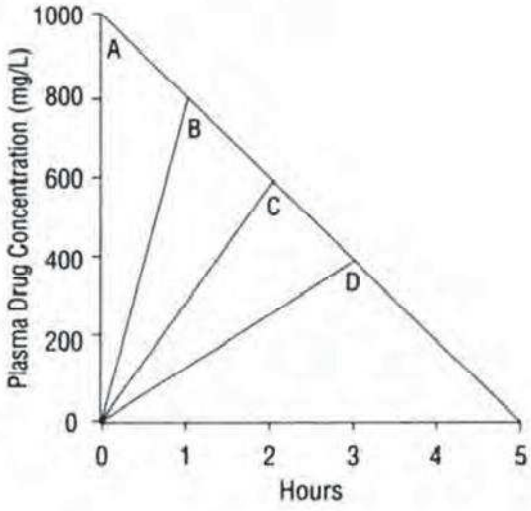


Fig. 22–12. A decrease in the absorption rate of alcohol, produced by slowing gastric emptying, causes peak concentrations and *AUC* to decrease and time to reach the peak to increase. The effect differs from that expected of first-order kinetics by the observed decrease in *AUC*. This observation is explained by a constant rate of elimination at almost all concentrations, as illustrated schematically in Fig. 22–13. Alcohol, 45 mL of 95% ethanol in 105 mL of orange juice, was administered 10 min after 240 mL of tap water (●); 240 mL of light cream (◆); or 240 mL of a 33% glucose solution (■) (1 mg/L = 22.0 μM). (Redrawn from data of Sedman, A.J., Wilkinson, P.K., Sakmar, E., Weidler, D.J., and Wagner, J.G.: Food effects on absorption and metabolism of alcohol. Reprinted by permission, from Journal of Studies on Alcohol, Vol. 37, pp. 1197–1214, 1976. Copyright by Journal of Studies on Alcohol, Inc., Rutgers Center of Alcohol Studies, New Brunswick, NJ 08903.)

Fig. 22–13. As a consequence of zero-order elimination, the plasma concentrations at the end of a 50-g dose of a drug by bolus injection (A) and constant-rate infusions of 1- (B), 2- (C), and 3- (D) hr durations are quite different from those expected with first-order kinetics. The amount in the body at the end of each infusion is the difference between dose and amount lost during the infusion period. Consequently, the concentration at the end of each of the infusions is the same as that expected at that time following the i.v. bolus dose. Note that the slower the input rate, the smaller is *AUC* and the lower is peak concentration. The time to peak is, of course, increased. Furthermore, if the dose had been infused over a 5-hr period, i.e., at 10 g/hr, output would have matched input and there would have been no *AUC* in this hypothetical example.



total, rather than unbound, concentration. Since *fu* is typically 0.1, the apparent *Km* for total concentration, *Km'*, is equal to 4 mg/L.

Plateau. Perhaps the most striking consequence of the kinetics of this drug is the relationship observed between steady-state plasma concentration and rate of administration, as shown in Fig. 22–14. A greatly disproportionate increase in concentration is observed in, and above, the therapeutic concentration range, 10 to 20 mg/L. As a result, the difference between the daily dose giving ineffective therapeutic concentrations, less than 10 mg/L, and that producing potentially toxic concentrations, above 20 mg/L, is narrow.

The observed increase in concentration can be explained by rearrangement of Eq. 8.

$$\frac{Cu_{ss}}{Km} = \frac{C_{ss}}{Km'} = \frac{R_o}{Vm - R_o} \qquad 9$$

The consequences of Michaelis-Menten metabolism result when either the desired steady-state unbound concentration is above *Km* (*Km'* for total concentration) or the rate of administration required to achieve these concentrations approaches *Vm*.

Because of its kinetics, only small changes in phenytoin input caused, for example, by a change in salt form (acid and sodium salt are used) or in bioavailability can produce relatively large changes in the steady-state concentration. To illustrate this point, consider a male patient with *Km'* and *Vm* values of 3 mg/L and 425 mg/day, respectively, and who has an average steady-state concentration of 12 mg/L when taking 200 mg orally every 12 hr. On switching from his current dosage form (bioavailability = 0.85) to one with a bioavailability of 0.95, it is seen, by setting $R_o = F \cdot D/\tau$ in Eq. 9, that the average steady-state concentration is expected to increase to 25 mg/L. Thus, a minor change in bioavailability (0.85 to 0.95) causes a major change (12 to 25) in the steady-state concentration when the dosing rate approaches the *Vm* value.

Time to Plateau. Because of capacity-limited metabolism, the time to reach steady state varies with the rate of administration. Figure 22–15 shows the approach to plateau during each of four dosing rates, which increase by small increments from 300 to 425 mg/day, in a patient with typical *Vm* and *Km'* values. Note that the time to reach 90% of plateau increases progressively with the rate of administration. These disproportionate changes in the steady-state concentration and the time required to reach them are major problems in optimally dosing phenytoin and interpreting its concentrations. Even though

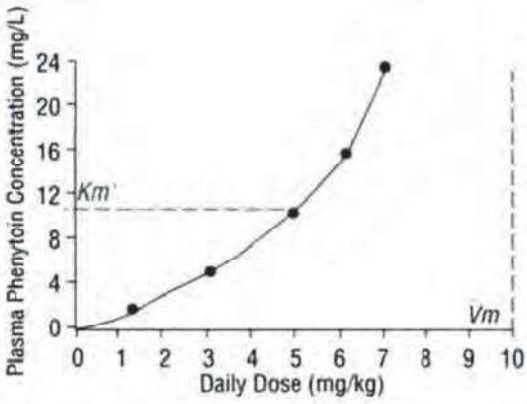


Fig. 22–14. The steady-state plasma concentration increases disproportionally with rate of administration (given twice daily) of phenytoin, a drug that is virtually eliminated by a single metabolic pathway and that exhibits typical Michaelis-Menten enzyme kinetics. The estimated *Vm*, the maximum rate of metabolism, and *Km'*, the total plasma concentration at which the rate is half of the maximum (colored line), are shown. All the data were obtained in the same individual whose *Km'* and *Vm* values are considerably higher than the typical ones of 4 mg/L and 7 mg/kg/day. (1 mg/L = 4.0 μM.) (Adapted from Martin, E., Tozer, T.N., Sheiner, L.B., and Riegelman, S.: The clinical pharmacokinetics of phenytoin. J. Pharmacokinet. Biopharm., 5:579–596, 1977. Reproduced with permission of Plenum Publishing Corp.)

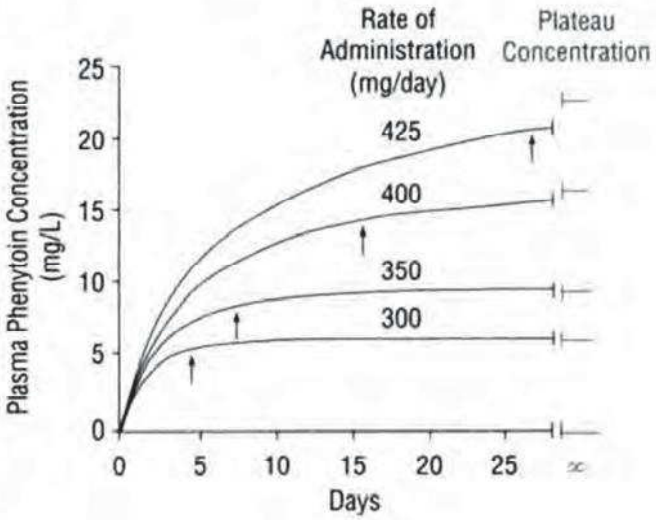


Fig. 22–15. Following administration (i.v. infusion is simulated) of phenytoin at constant rates of 300, 350, 400, and 425 mg/day, the plasma concentration approaches steady-state values of 6, 9.3, 16, and 22.7 mg/L, respectively (colored lines). Not only are the steady-state concentrations disproportionately increased, but so is the time required to approach the plateau. The arrows indicate the time required to reach 90% of the plateau value. The following parameter values were used: *Km'*, 4 mg/L; *Vm*, 500 mg/day; *V*, 50 L. (1 mg/L = 4.0 μM). (Reproduced by permission of publisher. Redrawn from Winter, M.E., and Tozer, T.N., Phenytoin, Chap. 25, in Applied Pharmacokinetics: Principles of Therapeutic Drug Monitoring, 3rd edition, edited by W.E. Evans, J.J. Schentag, and W.J. Jusko, published by Applied Therapeutics, Inc., Spokane, Washington, 1992.)

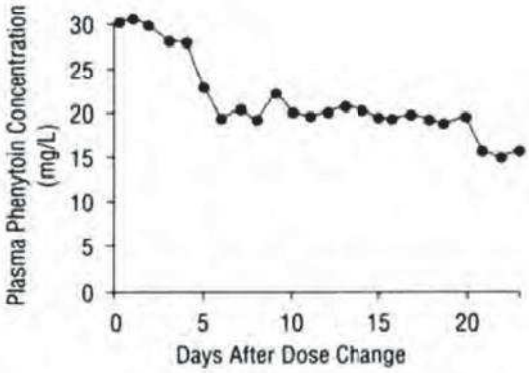


Fig. 22–16. On decreasing the daily dose from 250 to 200 mg/day, the plasma phenytoin concentration (obtained daily just before the morning dose) declines slowly toward a new steady state. Note that a 20% reduction in the daily dose leads to a 50% decrease in the concentration at steady state (the concentration stabilized after Day 23) (1 mg/L = 4.0 μM). (Redrawn from Theodore, W.H., Qu, Z.-P., Tsay, J.-Y., Pitlick, W., and Porter, R.J.: Phenytoin: The pseudosteady-state phenomenon. Clin. Pharmacol. Ther., 35:822–825, 1984. Reproduced with permission of C.V. Mosby.)

the approach to plateau is usually somewhat quicker on reducing doses than on increasing them, it can still take a long time, as shown in Fig. 22–16.

Because clearance, and hence half-life, are functions of the plasma concentration, the meanings of these parameters are lost when capacity-limited metabolism occurs. For this reason, these parameters should not be used for predicting or summarizing the kinetics of drugs showing this kind of behavior. The parameters of choice are those of the appropriate nonlinear model (*Km* and *Vm* for Michaelis-Menten kinetics and *V*).

Alterations in Metabolism. Another therapeutically important facet of the kinetics of phenytoin is altered metabolism brought about by other drugs and disease states. Either *Km'* or *Vm* can be altered, but the effect on plasma concentration is different. From Eq. 9 it can be seen that the phenytoin concentration at steady state is directly proportional to *Km'*. Thus, a condition such as competitive inhibition of metabolism, which affects the *Km'* value, produces corresponding changes in the steady-state phenytoin concentration.

For example, when cimetidine inhibits phenytoin metabolism, thereby increasing Km' from 4 to 6 mg/L, the steady-state unbound and total phenytoin concentrations are expected to increase by 50% as well.

In contrast, either an increase in Vm, brought about by enzyme induction, or a decrease in Vm, caused by the presence of hepatic cirrhosis, is expected to produce a disproportional change in the steady-state phenytoin concentration. This is seen by taking the ratio of the two different concentrations, $Cu_{ss,1}$ and $Cu_{ss,2}$ (Eq. 9), that result from the unaltered, Vm_1, and altered, Vm_2, values, respectively.

$$\frac{Cu_{ss,2}}{Cu_{ss,1}} = \frac{Vm_1 - R_o}{Vm_2 - R_o} \qquad 10$$

For example, when R_o = 300 mg/day, Vm_1 = 500 mg/day, and Vm_2 = 400 mg/day, the unbound concentration at steady state is doubled, $Cu_{ss,2}/Cu_{ss,1} = 2$. If Vm_2 is 600 mg/day, the ratio is 0.67. Thus, a 20% decrease in Vm doubles the steady-state concentration; whereas a 20% increase in Vm reduces the steady-state concentration by 33%. Note that a Vm of 300 mg/day results in a concentration approaching infinity, and that with a Vm below 300 mg/day, steady state can never be achieved. The input rate would then always exceed Vm, and Eqs. 5 to 10 would not be applicable.

Alcohol and phenytoin represent extreme cases in that almost all the elimination of each drug occurs by a single saturable pathway. More commonly, a drug is metabolized by several pathways, and only one or two of them approaches saturation. Then, saturation has less effect on total clearance. The extent of the effect depends on fm, the fraction of drug eliminated by the saturable pathway at low drug concentrations. Only if fm is 0.5, or greater, under nonsaturating conditions is total clearance materially affected by saturation of the pathway. Examples of drugs in this category include propranolol, theophylline, and salicylic acid.

TIME-DEPENDENT DISPOSITION

The study of changes in response to drug administration or kinetics with time of day, month, or year is an area called *chronopharmacology*. The pharmacokinetic component is *time-dependent kinetics* or *chronopharmacokinetics*. Carbamazepine shows time dependence in its disposition (Chap. 23, p. 430). The decrease in its peak concentration on repetitive oral administration indicates that either oral bioavailability decreases or clearance increases with time. The latter has been shown to explain the observation caused by carbamazepine inducing its own metabolism. This *autoinduction* is also dose- and concentration-dependent, a property common to many time-dependent processes.

Autoinduction has a number of therapeutic consequences. It affects the time to achieve steady state and limits one's ability to use information from a single dose to predict kinetics after repeated doses or continuous administration. Furthermore, it may be associated with the induction of metabolism (same enzymatic pathway) of coadministered drugs, producing a drug interaction.

Although enzyme induction is perhaps the most common cause of time-dependent kinetics, there are many other reasons for this behavior. For example, diurnal variations in renal function, urine pH, α_1-acid glycoprotein concentration, gastrointestinal physiology (food and drink), and cardiac output all occur. Chronic effects of a drug on its own renal and hepatic elimination have also been seen, as previously mentioned.

Aminoglycosides can produce renal toxicity with chronic administration. Because these antibiotics are primarily eliminated by renal excretion, a diminishing renal function with time may cause greater drug accumulation and therefore more toxicity. There is clearly a

need to monitor therapy and to limit the duration of therapy, especially in patients who already have compromised renal function.

An example of diurnal changes in drug absorption and disposition is shown in Fig. 22–17. Median data are shown (because of skewed distribution of values) for eight subjects who received an oral 80-mg dose of verapamil at various times during the day. Note that administration in the evening produces a lower peak drug concentration and, as such, may produce less effect than does administration in the morning.

Food is a major cause of diurnal variations. Gastric emptying is slowed or delayed by food (see Fig. 9–7), often resulting in a decrease in the peak concentration and an increase in the time of its occurrence following a single dose.

NONLINEAR METABOLITE FORMATION OR ELIMINATION

Nonlinear formation or elimination of a metabolite is of therapeutic interest when it accounts for some or all of either the therapeutic response, or toxicity, or both. The consequences of such nonlinearities depend on the cause and the site of occurrence. At the end of Chap. 21, the ratio of *AUC* values for metabolite and drug was explored as a means of evaluating changes in drug metabolism from one occasion to another. The ratios can also be used to assess sources of dose dependence.

Table 22–6 lists kinetic changes expected for a drug that forms two sequential metabolites following a single i.v. dose. Using the principles given in Chap. 21, the consequences of capacity-limited metabolism at each of the sites in the metabolic scheme can be assessed. For a compound with nonlinear metabolism, *AUC* increases with respect to the dose administered; the *AUC* of the product of the nonlinear pathway decreases with respect to the *AUC* of its precursor. If the site of nonlinearity involves the first of three consecutive steps, the ratio of areas of the last two products remains constant, though both areas may be decreased with respect to the dose given.

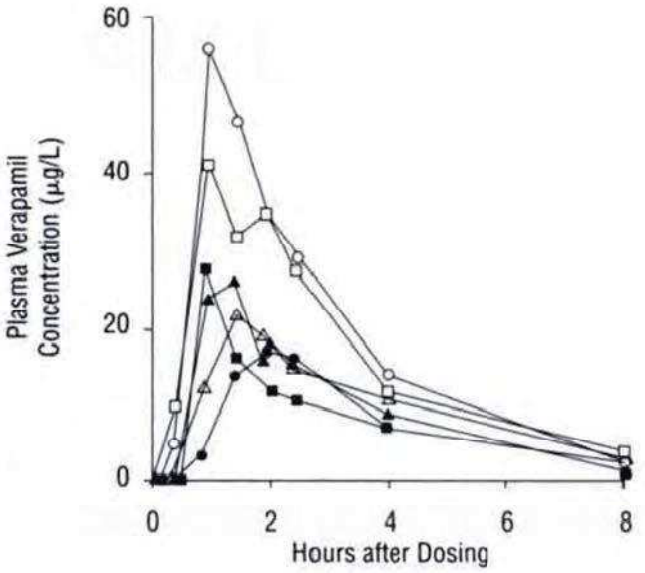


Fig. 22–17. Median plasma verapamil concentration-time profiles for eight subjects, each given a single 80-mg tablet of drug at the following times: 4:00 A.M. (▲); 8:00 A.M. (○); noon (□); 4:00 P.M. (△); 8:00 P.M. (●), and midnight (■). Food was withheld 2 hr before to 2 hr after drug administration, as food is known to affect verapamil absorption. To minimize a delay in esophageal transit, subjects took the tablets while standing and remained so for 15 min. The kinetics of the drug appears to change with the time of administration during the day. Factors other than food and posture may be responsible, e.g., changes in hepatic blood flow (verapamil has a high hepatic extraction ratio), enzyme activity, or protein binding (1 mg/L = 2.2 μM). (From Hla, K.K., Latham, A.N., and Henry, J.A.: Influence of time of administration on verapamil pharmacokinetics. Clin. Pharmacol. Ther., *51*:366–370, 1992.)

The general tendencies for changes in steady-state concentrations following chronic dosing are the same as those for *AUC* after a single dose. Here, however, the compound (drug or metabolite) with capacity-limited metabolism may not reach steady state if it is formed at a rate that exceeds its Vm.

Dose-dependence is expected in the degree of autoinduction for drugs that undergo this behavior. Such is observed with carbamazepine. As shown in Fig. 22–18A, the steady-state concentration of carbamazepine fails to increase in direct proportion to daily dose ($C_{ss,av}/(D/\tau)$ decreases). Furthermore, the ratio of the steady-state concentrations of the metabolite carbamazepine-10,11-epoxide and carbamazepine increase with dose (Fig. 22–18B). These results demonstrate nonlinearity in the formation of the metabolite. The directions of the changes suggest that formation clearance of the epoxide is increased.

RECOGNITION OF NONLINEARITIES

Nonlinearities are often apparent in graphic and tabular presentations of data. The interpretation of such kinetic behavior often requires a methodical analysis of the information. This analysis may be accomplished using the following steps:

1. Compare the observation to that expected for linear kinetics.
2. Identify the kinetic parameter(s) that appear(s) to be altered and the direction of the change.
3. Determine the primary pharmacokinetic parameter(s) CL_H, CL_R, V, ka, and F that appear to be affected. Also evaluate if fu is altered.
4. Consider the mechanism(s) consistent with the changes observed.

Urinary Recovery

Nonlinearities are often first identified from recovery of drug and metabolites in urine. This is the case for salicylic acid, as shown in Table 22–7.

Analysis

Step 1. Dose dependence in salicylic acid is apparent from changes in the fraction of a single oral dose recovered as unchanged drug and as the metabolites, salicyluric acid and glucuronide conjugates. With linear kinetics, the fraction of each compound recovered remains the same regardless of dose.

Table 22–6. Consequences of Capacity-Limited Processes in the Sequential Metabolism of a Drug Given in a Single Dose

MODEL	$\frac{AUC}{DOSE}$	$\frac{AUC(m_1)}{AUC}$	$\frac{AUC(m_2)}{AUC(m_1)}$
1. $D \rightarrow M_1 \rightarrow M_2 \rightarrow$ $\downarrow$	$\leftrightarrow$[a]	$\leftrightarrow$	$\leftrightarrow$
2. $D \Rightarrow$[b] $M_1 \rightarrow M_2 \rightarrow$ $\downarrow$	$\uparrow$[c]	$\downarrow$	$\leftrightarrow$
3. $D \rightarrow M_1 \Rightarrow M_2 \rightarrow$ $\downarrow$	$\leftrightarrow$	$\uparrow$	$\downarrow$
4. $D \rightarrow M_1 \rightarrow M_2 \Rightarrow$ $\downarrow$	$\leftrightarrow$	$\leftrightarrow$	$\uparrow$
5. $D \rightarrow M_1 \rightarrow M_2 \rightarrow$ $\Downarrow$	$\uparrow$[c]	$\leftrightarrow$	$\rightarrow$

[a] $\leftrightarrow$, little or no change; $\uparrow$, increase; $\downarrow$, decrease on increasing the dose.
[b] ie, capacity-limited metabolism of Michaelis-Menten type.
[c] The affected pathway is the major route of elimination at nonsaturating doses. Otherwise, little change is expected.

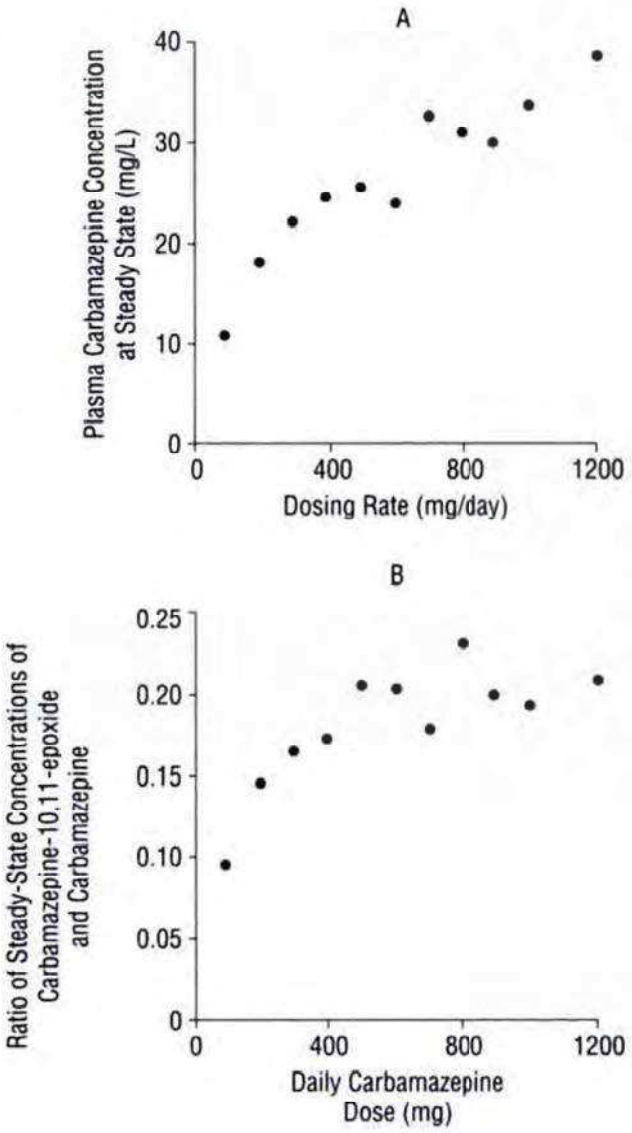


Fig. 22–18. A, The steady-state concentration of carbamazepine fails to increase in direct proportion to the daily dose, evidence of nonlinear kinetics. *B*, The ratio of the concentrations of the metabolite, carbamazepine-10,11-epoxide, and carbamazepine increase with dosing rate. These observations indicate that either the clearance of formation increases or the clearance of elimination of the epoxide decreases at higher dosing rates. The former, a consequence of dose-dependent autoinduction of metabolite formation, is the explanation. Each point in both graphs is the mean of data from 77 patients. (Carbamazepine: 1 mg/L = 4.2 μM; carbamazepine 10,11-epoxide: 1 mg/L = 4.0 μM.) (Adapted from Kudriakova, T.B., Sirotsa L.A., Rozova, G.I., and Gorkov, V.A.: Autoinduction and steady-state pharmacokinetics of carbamazepine and its major metabolites. Br. J. Clin. Pharmacol., 33:611–615, 1992.)

Table 22–7. Urinary Recovery of Salicylic Acid and its Metabolites as a Percent of a Single Oral Dose[a]

DOSE (mg)	SALICYLIC ACID	SALICYLURIC ACID	SALICYL PHENOLIC AND ACYL GLUCURONIDES
192	3	83	17
767	5	70	24
1533	17	59	24
3000	14	50	30

[a]Dose and recovery expressed in equivalents of salicylic acid. (Adapted from Levy, G.: Pharmacokinetics of salicylate elimination in man. J. Pharm. Sci., 54:959–967, 1965.)

Steps 2 to 4. The increased percent recovered as unchanged drug on increasing dose could be explained by an increased bioavailability, an increased renal clearance, or a capacity limitation in its metabolism. This is seen from the relationships $Ae_\infty/\text{Dose} = fe \cdot F$ and $fe = CL_R/(CL_R + CL_{NR})$. Without further information, these possibilities cannot be distinguished. The decreased recovery of the major metabolite, salicyluric acid, and the nearly complete total recovery (as drug and metabolites) at all of the doses suggest that increased bioavailability may not be the explanation (see Chap. 21). Increased bioavailability would increase metabolite recovery as well, which was not the case.

Concentration-Time Profile

Figure 22–19 is a semilogarithmic plot of plasma salicylic acid concentration with time after a single 3-g oral dose.

Analysis

Step 1. The slope of the decline is greater at low than at high concentrations. When linear kinetics operates, there is either no change in slope or a greater slope at early times while distribution is occurring (Chap. 19, Distribution Kinetics).

Step 2. The half-life shortens as time passes or as concentration declines.

Step 3. It appears that either CL increases or V decreases with time or with falling concentration.

Step 4. From the dose and maximum concentration (around 200 mg/L) and the conclusion based on urinary data (and independent i.v. data) that salicylic acid is totally absorbed after oral administration, the volume of distribution is estimated to be about 15 L. Being so small, V is unlikely to decrease much with either time or decreasing concentration. An increase in CL with time or at lower concentrations would occur if elimination of salicylic acid is capacity-limited. The data in Table 22–7 indicate that salicylic acid is extensively

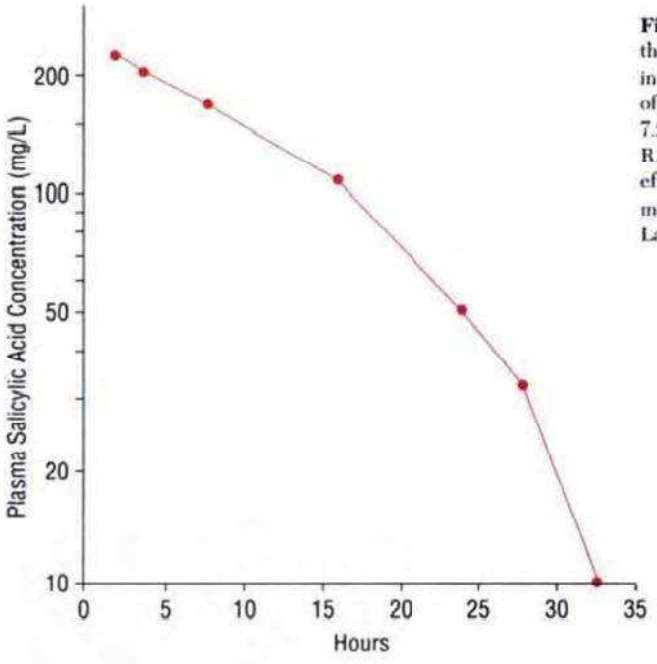


Fig. 22–19. Semilogarithmic plot of the plasma concentration of salicylic acid in a subject following a single oral dose of 3 g of sodium salicylate (1 mg/L = 7.2 μM). (Adapted from data in Salassa, R.M., Bollman, J.L., and Dry, T.J.: The effect of para-aminobenzoic acid on the metabolism and excretion of salicylate. J. Lab. Clin. Med., 33:1393–1401, 1948.)

converted to several metabolites. However, the pronounced increase in fraction excreted as unchanged salicylic acid with increasing dose (Table 22–7) implies that saturability of a major metabolic pathway is likely at the 3-g dose level.

Protein Binding Data

The incorporation of binding data further clarifies the nature of nonlinearities present, as shown in Fig. 22–20.

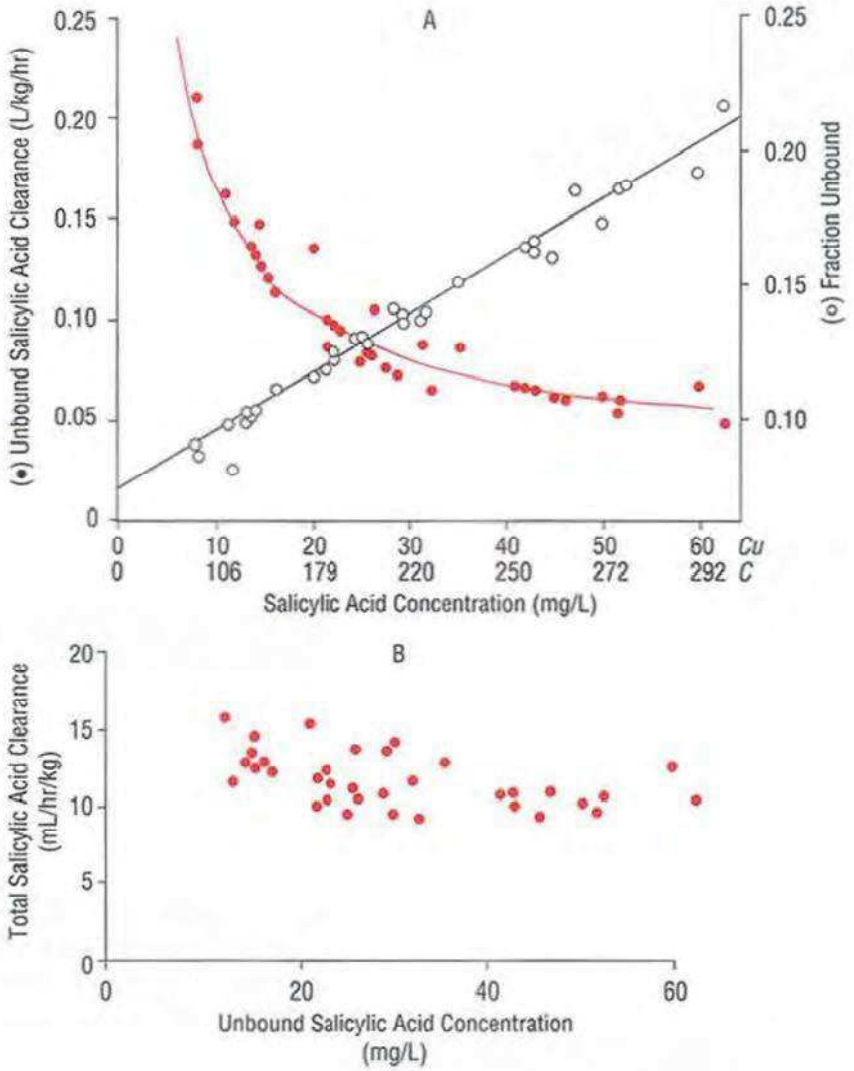


Fig. 22–20. *A*, The clearance of unbound drug (●, colored line), determined under steady-state conditions, and the fraction unbound in plasma (○) vary inversely with each other as the salicylic acid concentration is increased. The corresponding total plasma concentrations are superimposed on the linear scale of the unbound drug concentration (1 mg/L = 7.2 μM). (Redrawn from Furst, D.E., Tozer, T.N., and Melmon, K.L.: Salicylate clearance: The result of protein binding and metabolism. Clin. Pharmacol. Ther., 26:380–389, 1979. Reproduced with permission of C.V. Mosby.) *B*, The total clearance of salicylic acid, determined under steady-state conditions, remains essentially constant within the range of therapeutic concentrations; a fortuitous consequence of the essentially equivalent and opposing effects of saturable plasma protein binding and saturable metabolism (1 mg/L = 7.2 μM).

Analysis

Step 1. As observed in Fig. 22–20A, *fu* increases with increasing concentration in the antirheumatic therapeutic range, 100 to 300 mg/L, indicating nonlinearity of salicylic acid binding to plasma proteins. Nonlinearity in *CLu* is also uncovered. As shown in Fig. 22–20B, salicylic acid has a low clearance even at low concentrations. As such, *CLu* should remain constant if elimination processes are unaffected by the concentration (dose) of salicylic acid.

Steps 2–3. The parameters affected are identified in this case, *fu* and *CLu*.

Step 4. The mechanisms involved appear to be saturable binding to plasma proteins and some form of capacity-limited elimination. From the additional information in Table 22–7 and Fig. 22–19, the capacity limitation must lie with metabolism.

Interestingly, total clearance is relatively constant throughout the therapeutic range of salicylic acid (*Cu* between 10 and 60 mg/L). This is a consequence of the opposing tendencies of the two forms of nonlinearity. Clearance decreases with concentration because of capacity-limited metabolism. Saturable binding, on the other hand, tends to increase clearance at higher concentrations, $CL = fu \cdot CLu$. The decrease in unbound clearance, the consequence of saturability in two major metabolic pathways of salicylic acid (formation of salicyluric acid and salicyl phenolic glucuronide) leads to a disproportionate increase in unbound concentration with increasing dosing rate (Fig. 22–21). Further information on the specific nonlinear metabolic pathways requires isolating the metabolic pathways. This has been accomplished by comparing the rates of urinary excretion of each metabolite to the amount of salicylic acid in the body, a procedure analogous to the determination of renal clearance. The method is applicable if the rate of excretion of metabolite equals its rate of formation, as is the case for salicylic acid after a single dose and for all drugs under steady-state conditions.

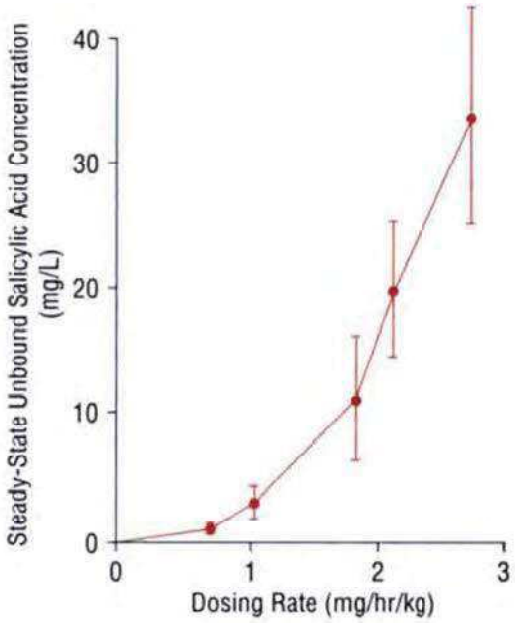


Fig. 22–21. The steady-state unbound concentration of salicylic acid in plasma (mean ± SD) increases disproportionately with the dosing rate of aspirin. Aspirin is completely converted to the measured metabolite, salicylic acid. The disproportionate increase reflects the saturability of two of salicylic acid's major pathways of elimination, formation of salicyl phenolic glucuronide and salicyluric acid (1 mg/L = 7.2 μM). (Redrawn from Tozer, T.N., Tang-Liu, D.D.-S., and Riegelman, S.: Linear vs. nonlinear kinetics. In Topics in Pharmaceutical Sciences. Edited by D. Breimer and P. Speiser. New York, Elsevier, 1981, pp. 3–17.)

THERAPEUTIC CONSEQUENCES

The therapeutic consequences of dose-dependent kinetics are perhaps best considered under steady-state conditions. Here, dose dependence in rate-time profile of absorption is of little or no consequence unless bioavailability (extent) is also altered. Alterations in bioavailability are important in that unbound concentration and amount in the body at steady state may change disproportionately on changing dose. Conditions that cause dose dependence in bioavailability may also produce increased variability in this parameter at the same dose. For example, consider two drugs, a dissolution rate-limited one, such as griseofulvin, and one that is highly extracted on passing across the gastrointestinal membranes or through the liver, such as alprenolol. Changes in gastric emptying and in other physiologic factors can produce large variations in the bioavailability of these drugs. The effect of rapid gastric emptying may be a decrease in bioavailability of griseofulvin because of less time for mixing and dissolution but an increase in bioavailability of alprenolol because of saturability in intestinal and hepatic metabolism.

Clinically, the most dramatic source of dose dependence is capacity-limited metabolism. Only small changes in bioavailability may produce large changes in the steady-state concentration. Under these conditions, careful titration of an individual patient's dosage requirement is needed, especially if the drug has a narrow therapeutic index. Moreover, this source of dose-dependence also produces large interpatient variability in steady-state concentration, as observed for phenytoin in Fig. 1–5.

Time-dependent kinetics is perhaps best typified by autoinduction. Another cause is decreased renal function on continued administration of a nephrotoxic drug. If the drug is primarily renally eliminated, the therapeutic consequence of the latter cause is clear and opposite to that of autoinduction.

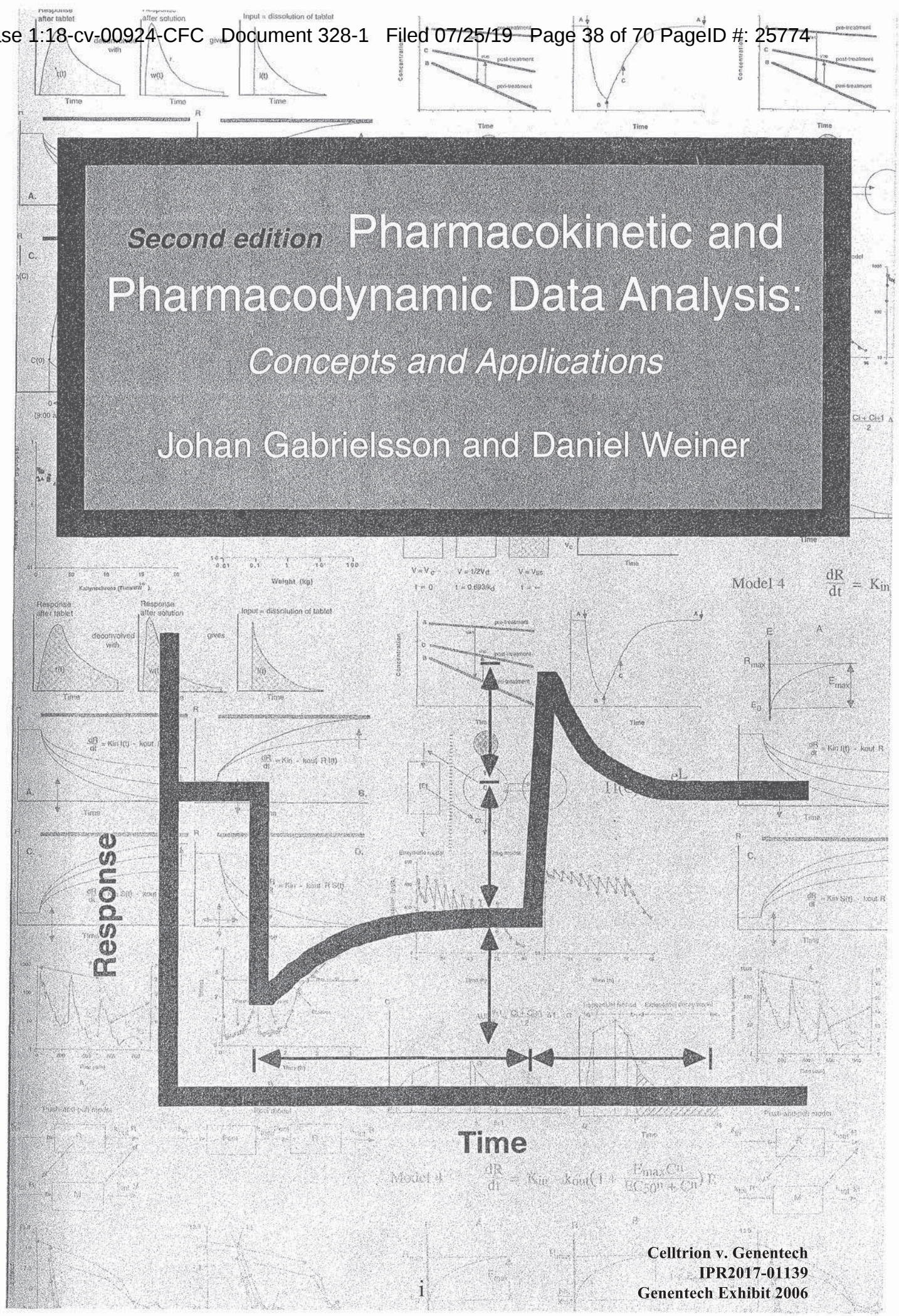
In general, if two drugs are equivalent in all respects except for one showing either a dose- or time-dependent kinetic behavior, then the one showing linear kinetics is the drug of choice.

STUDY PROBLEMS

(Answers to Study Problems are in Appendix II.)

1. Circle the number(s) of the one or more *correct* answers to each of the following multiple-choice questions. Briefly discuss those answers that may be ambiguous (situations in which the statement may be true or false).
   a. Capacity-limited metabolism of a drug is associated with:
      1. A less than proportional increase in the steady-state plasma concentration on increasing the total daily dose.
      2. An apparent half-life that is longer at low than at high concentrations.
      3. A decrease in the rate of metabolism as the amount of drug in the body is increased.
      4. All of the above.
      5. None of the above.
   b. A mean steady-state plasma concentration obtained after 30 doses that is much higher than that predicted from the value of clearance obtained after a single dose suggests that:
      1. Induction has occurred.
      2. The volume of distribution has decreased.
      3. A metabolite may be acting as an inhibitor of drug elimination.
      4. None of the above.

EXHIBIT 232



Second edition Pharmacokinetic and Pharmacodynamic Data Analysis:

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$$\text{Rate} = \frac{V_{max}\, C}{K_m} = K' C \qquad (3:186)$$

and V_{max} cannot be separated from K_m. This may also be appreciated from Figure 3.51. V_{max}/K_m is the slope of the linear portion of the curve at concentrations less than K_m.

In order to estimate V_{max} and K_m accurately we need to measure plasma concentrations that cover the majority of the curvature. To estimate K_m accurately and separated from the V_{max} estimation, one needs concentration information from K_m and below. That may be done by fitting the kinetic model simultaneously (including the Michaelis-Menten like elimination rate) to both high-dose and low-dose plasma concentration-time data. V_{max} and K_m may then be estimated separately with a sufficient amount of accuracy and precision and relatively low correlation.

For a one-compartment system following iv dosing with Michaelis-Menten elimination the area-under-the-curve can then be estimated as

$$AUC = \frac{V_{max}\, C^0}{2}\left[K_m + \frac{C^0}{2}\right] \qquad (3:187)$$

where C^0 is the concentration at $t = 0$. Capacity limited kinetics may be summarized in Figure 3.52.

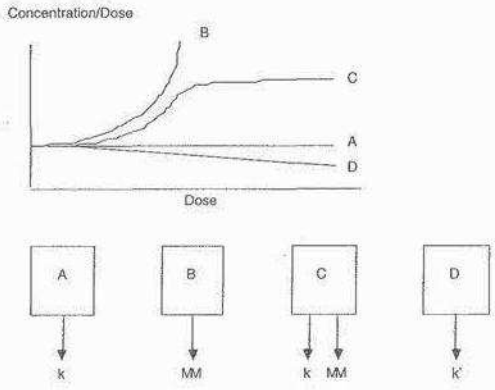

Figure 3.52 *Dose-normalized concentration versus dose for a linear system (A), capacity limited elimination (B) and parallel linear and capacity limited system (C). System D is a pseudo-second order system. System C is exemplified by salicylic acid and system D by e.g., albumin.*

The half-life is a function of plasma concentration for the nonlinear system. It is not the time required to eliminate one-half of the drug in the body, but is rather an instantaneous value of the time required to eliminate half of the drug present, if the fractional rate of elimination were to continue at that value (Tozer and Winter [1992]).

$$t_{1/2} = \ln(2)\frac{V_{ss}}{V_{max}}(K_m + C) \qquad (3:204)$$

Since clearance and half-life are first-order pharmacokinetic parameters, the most useful parameters for a nonlinear system are V_{max}, K_m and V_{ss}. The authors pointed out that in the case of phenytoin, the importance of further research of the time dependency, stability, of V_{max}, K_m and V_{ss}. One would expect enzyme induction to increase V_{max} but to have no effect on K_m. One would expect K_m, based on the unbound concentration, to be relatively constant, because it depends on the property of the enzyme. For a comprehensive review on nonlinear (phenytoin) pharmacokinetics see Tozer and Winter (1992). More information on how to derive initial parameter estimates of K_m and V_{max} for the one- and two compartment models are presented in the applications section on nonlinear (capacity limited) kinetics (Section PK20).

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EXHIBIT 243

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

AMGEN INC.; AMGEN MANUFACTURING, LIMITED; and AMGEN USA INC. Plaintiffs, v. SANOFI; SANOFI-AVENTIS U.S. LLC; AVENTISUB LLC, f/d/b/a AVENTIS PHARMACEUTICALS INC., and REGENERON PHARMACEUTICALS, INC., Defendants.	C.A. No.: 14-1317-SLR (CONSOLIDATED) [redacted] PUBLIC VERSION

PLAINTIFFS' OPENING BRIEF IN SUPPORT OF
MOTION FOR PERMANENT INJUNCTIVE RELIEF

[redacted]

1. Defendants Pursued Praluent® Despite Knowledge of the Infringement Risk and Then Launched At-Risk During the Pendency of This Litigation

Defendants were aware of the potential infringement risk when they chose to pursue Praluent® and should not now be permitted to avoid an injunction by relying on the harms suffered when this business decision did not pan out in their favor.[10]

As early as February 2009, Defendants knew of Amgen's published patent application and immediately identified multiple risks. Hr'g Tr. 187:18-188:7 (Papadopoulos designations). First, the PCT included the full specification of the patents-in-suit as well as both broad ***and*** specific claims that would have covered Defendants' REGN727, which Defendants knew and understood. *See* PTX 3888 at -232 (*see* claims 1, 7, 8, 9); *see* Hr'g Tr. 378:10-15 (Stahl cross). Second, by June 2009, Defendants had identified the risk of a "patent issue" if the "binding epitopes" between REGN727 and Amgen's antibodies were "similar." Hr'g Tr. 191:7-22 (Papadopoulos designations); PTX 3965. Defendants then confirmed that was the case when they discovered overlap in the epitopes between REGN727 and Amgen's antibodies. Hr'g Tr. 188:22-25, 192:20-194:2 (Papadopoulos designations); PTX 3972 at -755; PTX 3957; *see also* Hr'g Tr. 376:19-378:15 (Stahl cross); PTX 3880.

Defendants then performed x-ray crystallography studies for the express purpose of assessing whether Praluent® bound to the PCSK9 residues identified in Amgen's patent

[10] At the hearing, Defendants suggested that Amgen was re-arguing willfulness in support of its injunction position. Hr'g Tr. 38:20-24 (Maslowski opening). Not so. The Court need not reach issues of intent or objective or subjective reasonableness or belief. What the uncontested evidence showed was that Defendants took a calculated business risk, despite Amgen's patents, to press forward with their investment and, ultimately the launch of Praluent®. It does not matter whether they took that risk with a good faith belief that Amgen's patents were not valid. What matters is that they took the risk knowing that Amgen was seeking patent protection and that Amgen's patents might well be found to be valid and, in that event, Defendants' infringing product might be staring at an injunction. *See Acumed*, 551 F.3d at 1330.

application, which itself suggests that Defendants understood which residues would be important from a patent perspective, even without issued claims. Hr'g Tr. 199:12-203:9 (Engel designations); PTX 6077 at -792, PTX 6131 at -522-23; Hr'g Tr. 378:16-21 (Stahl direct). In fact, Dr. Engel's study of the PCSK9 residues at the PCSK9/LDLR interface as set forth in Amgen's patent application (*see* PTX 6131 at -522-23, "projection of binding residues onto PCSK9 sequence: REGN727 . . . , Amgen 31H4, 21B12 *and LDLR-EGFa as described in patent WO 2009/026558A1*" (emphasis added)), are the same exact set of residues that became claim 1 of the '165 patent, which come directly from Example 28 in the patent. In sum, *each* experiment performed by Defendants demonstrates that they understood all along the risk that patent claims would issue directed to the PCSK9 residues at the LDLR interface and/or to which 21B12 and 31H4 bound. Notwithstanding Defendants' knowledge of Amgen's pending patent portfolio, as well as their own experimental evidence of infringement, Defendants elected to continue their investment in Praluent®. *See, e.g.*, Hr'g Tr. 342:20-343:8, 357:9-12 (Edelberg cross); PTX 4051 at -878.

Thus, Defendants' suggestion at the hearing that they should not be held accountable for their choices because Amgen's published patent applications might never have issued or might have issued with different claims should not be credited. Indeed, one of the very purposes behind enacting the legislation that mandates publication of patent applications is to provide notice to potential infringers. *See* Hr'g Before the Subcomm. on Cts. and Intellectual Property, 104th Cong. 36 (1995) (statement of Bruce Lehman, Asst. Sec. of Commerce and Comm'r of Patents and Trademarks, Patent and Trademark Office, U.S. Dept. of Commerce).[11]

[11] Available at: https://archive.org/details/patentslegislati00unit.

[redacted]

Even if the Court were to countenance Defendants' hubris in moving forward in the face of Amgen's patent application, Defendants' actions after this lawsuit was filed in October 2014 are telling. Defendants intentionally accelerated their infringement by exercising a priority review voucher to speed up the FDA approval process and their commercial launch by four months. Hr'g Tr. 61:2-13 (Bradway direct); Hr'g Tr. 174:1-13, 175:16-176:13 (Broadhurst direct); PTX 4530, PTX 4758. Defendants chose to launch Praluent® at-risk despite the fact that the Court discussed, and then implemented, an accelerated trial date in lieu of preliminary injunction proceedings. *See* Compl., D.I. 1; Tr. of Feb. 24, 2015 Rule 16 Scheduling Conference 12:14-24, 16:25-17:4; Scheduling Order, D.I. 49. Upon the launch of Praluent®, Defendants knowingly exacerbated the harm to Amgen by "flooding" the market with free goods and by adopting highly aggressive negotiation tactics to secure exclusive agreements with various insurers, including one of the largest insurance companies in the country. JTX 195 at -758; Hr'g Tr. 315:7-15; 316:3-20 (Terifay cross); Hr'g Tr. 166:20-169:19 (Broadhurst direct); Hr'g Tr. 226:16-227:6 (Berndt direct); Hr'g Tr. 285:8-287:12 (Carey cross); JTX 266 at -954. The logical inference drawn from Defendants' actions is that they chose to develop at-risk and launch at-risk — in order to argue that Praluent® should not be enjoined. Defendants are now asking the Court to reward them for their risky business decisions and their infringement. But such a reward would undermine the very purpose of obtaining a patent.

Courts have held that where infringers launched at-risk, any damage caused by this at-risk activity weighs against the infringer in the balance of the hardships analysis. *See, e.g., Merial Ltd. v. Cipla Ltd.*, 681 F.3d 1283, 1306 (Fed. Cir. 2012); *see also Robert Bosch LLC v. Pylon Mfg. Corp.*, 659 F.3d 1142, 1156 (Fed. Cir. 2011) (requiring a patentee to compete against its own patented invention places a substantial hardship on the patentee, weighing in favor of the

entry of an injunction); *Sanofi-Synthelabo v. Apotex, Inc.*, 470 F.3d 1368, 1383 (Fed. Cir. 2006) (affirming grant of preliminary injunction where generic drug manufacturer launched at-risk, stating that the balance of the hardships weighs in favor of the patentee where the infringer's hardships "were the result of its own calculated risk to launch its product pre-judgment"). The harms Defendants allege they will suffer simply do not outweigh the incalculable and irreparable harms inflicted upon Amgen due to Defendants' at-risk conduct.

2. Regeneron's Costs to Develop the Infringing Product Do Not Weigh in Defendants' Favor When Balancing the Hardships Because Those Costs Are Mitigated by Defendants' Collaboration Agreement

At the injunction hearing, Defendants signaled that they will claim harm because they spent time and money developing Praluent® and because Regeneron is a small biotech company. *See, e.g.*, Hr'g Tr. 481:3-6 (Oster direct). The decision to invest at-risk in development of Praluent® is addressed above. To the extent Defendants rely upon Regeneron being a "small" company, that argument fails. First, "[a] party cannot escape an injunction simply because it is smaller than the patentee or because its primary product is an infringing one." *Robert Bosch*, 659 F.3d at 1156; *see also Merial Ltd.*, 681 F.3d at 1306 (affirming entry of permanent injunction even where it would have a "crippling effect" on the infringer's business). Second, contrary to Defendants' assertion, Regeneron is by no means a small business and undoubtedly will continue due to its success as a pharmaceutical company, specifically with its Eylea® product, a self-proclaimed "best in class" drug for the treatment of macular degeneration, as well as other diseases of the eye. Trial Tr. 344:19-345:7 (Schleifer direct).

Third, if Praluent® is enjoined, Regeneron is shielded from significant harm due to its Collaboration Agreement with Sanofi. JTX 089 at §§ 9.1-9.12, Schedules 2 and 3; JTX 369 at §§ 4.1-4.10; Hr'g Tr. 237:22-238:22 (Berndt direct); Hr'g Tr. 312:10-18 (Terifay cross) (Sanofi

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CERTIFICATE OF SERVICE

The undersigned counsel hereby certifies that true and correct copies of the foregoing document were caused to be served on July 16, 2019 on the following counsel in the manner indicated:

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