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Studies on the Absorption of practically Water-insoluble Drugs following Injection. I. Intramuscular Absorption from Water-immiscible Oil Solutions in Rats

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The absorption behavior following intramuscular injection of practically water-insoluble drugs in water-immiscible oil solution was investigated with several azo dyes and two steroids as model drugs by the local clearance method in the *m. gastrocnemius* of the rat. The absorption of the drug component obeyed approximately 1st order kinetics, while the absorption of the oily solvent was very slow. The injection volume (V_0) influenced the absorption rate constant (k) and the correlation $k \propto V_0^m$ was experimentally observed (for intact rats, $m=-0.14$; for anesthetized rats, $m=-0.32$). This was also the case at another injection site, the *m. rectus femoris* in rats. Comparison of the absorption rate of a drug in various oil vehicles showed that k was controlled predominantly by the oil/water distribution coefficient (K) and depended little on the viscosity of the vehicle. These results suggested that the release process of the drug component from the oily depot to the aqueous phase around it was the main route for absorption, and that the subsequent transport process in the aqueous phase might be rate-limiting. A plot of log k versus log K gave a straight line with a slope close to -1, but of a slightly smaller absolute value. This plot could be applied satisfactorily for estimating the absorption rate of other drug-oil systems. A guideline for predicting the absorption rate of a drug in oily suspension from the k value for its solution is presented.

Keywords——drug absorption kinetics; intramuscular injection; local clearance method; practically water-insoluble drugs; water-immiscible oil vehicles; injection volume; distribution coefficient; intact and anesthetized rats

Parenteral routes of drug administration are as useful and important as oral ones for early screening and preclinical testing of drugs in animals. There are many kinds of injections, including intramuscular, subcutaneous, intravenous, intradermal, hypodermal, intraarterial, intrapleural, intraperitoneal, intraarticular, intracardial, intraspinal and intracerebral. Absorption is not involved when a drug is administered parenterally by an intravasular route. However, when the drug is administered by any extravascular route, a depot of some type is formed and the drug must leave the depot and reach the blood or lymph systems by some process(es).

Since the publication of several reviews[1] there has been increasing interest in the absorption behavior of drugs in aqueous solution from intramuscular[2] and subcutaneous sites.[3] However, compared to water-soluble drugs, little work has been done on practically water-insoluble drugs. These drugs are commonly administered to laboratory animals as parenteral preparations in the form of oily solutions (oily suspensions), aqueous suspensions, aqueous solutions solubilized with nonionic surfactants, or emulsions. The absorption of such drugs, which depends on the dosage form or formulation, is in most cases slow enough to be rate-determinant in their disposition in the body. This means that even pharmacological responses are sometimes governed primarily by the formulation itself. Therefore, basic investigation on drug absorption from such dosage forms by each parenteral route is required in order to select the optimal preparation and parenteral route for more complete screening tests in animals.

The present study was undertaken to elucidate the absorption behavior of practically water-insoluble drugs from water-immiscible oil solutions injected intramuscularly. Early

investigators[4] noted mainly the vehicle effect on the pharmacologic responses or blood levels of drugs but did not refer to the mechanism or kinetics of drug absorption from such preparations. Recently, Tanaka *et al.*[5] studied these problems using slightly water-soluble drugs in anesthetized and operated rats.

For our study, several azo dyes and some steroids which are unionized and practically water-insoluble under physiological conditions were used as model drugs, and their intramuscular absorption properties were examined by the local clearance method in the *m. gastrocnemius* of the intact rat. Under various experimental systems and conditions, the intramuscular absorption rates of these model drugs were compared to evaluate the contribution of physicochemical factors and to clarify the kinetic process. On the basis of the results, predictions of the absorption rates of other drugs in oily solutions were attempted. The relationship between absorption rates in oily solution and oily suspension was also investigated.

Experimental

Materials——Azo dyes such as *p*-aminoazobenzene (PAAB), *p*-hydroxyazobenzene (PHAB), *o*-aminoazotoluene (OAAT), 1-phenylazo-2-naphthylamine (PANA) and tetrazobenzene-β-naphthol (Sudan III), and steroids such as testosterone (TS) and 2α,3α-epithio-5α-androstan-17β-ol (epitiostanol) were selected as model compounds for practically water-insoluble drugs. These compounds (other than epitiostanol) were obtained commercially: PAAB, PANA and TS, Tokyo Kasei Kogyo Co., Ltd. (Tokyo); PHAB, Eastman Kodak Co. (N.Y.); OAAT, Ishizu Pharmaceutical Co., Ltd. (Osaka); Sudan III, E. Merck AG (Darmstadt). These compounds were of reagent grade and were used without further purification, except for PHAB, which was purified by being dissolved in MeOH then recrystallized with water. Epitiostanol was synthesized in our laboratory and was of medicinal grade. As water-immiscible oil solvents for the injection preparations, sesame oil (SO), medium chain (C_8–C_{12}) triglyceride commercially named Miglyol 812 (Mig), isopropyl myristate (IPM), diethyl sebacate (DES) and castor oil (CO) were selected in view of relatively wide variety in viscosity and dissolving powder. These compounds were also obtained from commercial sources: SO, Maruishi Pharmaceutical Co., Ltd. (Osaka); Mig, Chemische Werke Witten (Germany); IPM and DES, Nikko Chemicals Co., Ltd. (Tokyo); CO, Kenei Pharmaceutical Co. (Japan). SO and CO were of JP grade and the others were of reagent grade. All were used without further purification. All other chemicals used in this investigation were of analytical or reagent grade.

Test Injection Preparations——An oily solution was prepared by dissolving the desired amount of a test compound in an oily solvent and filtering it through an SM 116 membrane filter (Sartorius-Membranfilter GmbH, Göttingen). All test solutions used here were ascertained to be chemically and physically stable for at least the experimental period. An oily suspension of PAAB or TS was formulated in a room held at 37° by dispersing the sieved powder (for PAAB, 74—149 μm in diameter; for TS, 37—74 and 74—149 μm) in the oil vehicle presaturated with each drug using a mixer (Micro Thermo Mixer Model TM-101, Thermonics Co., Ltd., Tokyo), and the product was stored at 37° until use. All suspensions were used about 20 hr after preparation.

Animal Experiments——Male Wistar albino rats weighing 250±30 g were used in all animal experiments.

(i) Absorption Experiment Procedure: The injection site for the present study, unless otherwise mentioned, was the medial head of the *m. gastrocnemius* in the *m. triceps surae* of the left hind leg in the rat; this site was selected because the location of the needle tip could be easily controlled. The absorption time course was followed by the local clearance method. The rat was given light anesthesia with ether, then a thin Terumo needle (27G×3/4″ for oily solutions and 25G×1″ for oily suspensions; Terumo Co., Ltd., Tokyo) connected to a volume scale-corrected syringe or Terumo micrometer syringe (MS 10, 50 and 100) was inserted a few millimeters above the ankle and the tip of the needle was led to the center of the *m. gastrocnemius*, medial head. Next, the test solution (50 μl, unless otherwise stated) was injected at moderate speed. Immediately after withdrawal of the needle, the quick adhesive Aron Alpha (Toa Gousei Kagaku Kogyo Co., Ltd., Tokyo) was applied to the insertion site in order to prevent leakage of the oily solution injected. During the absorption experiment, the rat was housed in a cage which allowed free movement and easy access to water and food. At various intervals after the injection, rats were decapitated and bled, and the muscle tissues around the injection site, including the oily depot, were excised as completely as possible. The removed muscles were mixed with 5 ml of water and homogenized with a high speed blender (Ultra-Turrax, Janke and Kunkel K.G., Ger.) under cooling in an ice-water bath. Another 5 ml of water was used to wash the blender of the homogenizer and this was added to the above homogenate. After extraction from the homogenate with 8 ml of ethyl acetate, the residual amount of the drug in the removed muscles was analyzed. For absorption studies with sesame oil (SO), the following indirect method was adopted. Fifty microliters of SO solution containing Sudan III (5 mg/ml) was administered to the same site in the manner described above in two groups of rats. At a set time interval, both groups were sacrificed.

From one group, the residual amount (W) of Sudan III was determined, and from the other, its concentration (C) in SO solution remaining in the muscle was determined. Since Sudan III was pooled and kept homogeneously dissolved in the SO depot for at least 20 hr after injection, the volume (V) of SO remaining in the muscle could be approximately calculated as $V=W/C$.

(ii) *In Vitro* Incubation Experiment: In order to ascertain whether the clearance of a given drug from the injection site could be attributed to its transport into the vascular systems or not, an *in vitro* incubation experiment was undertaken along with the absorption experiment, and the metabolic change at the injection site was checked. Fifty microliters of SO solution containing 0.5 mg/ml of an azo compound was injected into the same site in the manner described above in two groups of rats. For one group, the muscles, including the injected solution, were removed immediately after administration, then incubated in 5 ml of Ringer-Locke solution under bubbling with air and mild shaking at 37° for 2 or 6 hr (Yamato incubator, model BT-41; Yamato Scientific Co., Ltd., Tokyo). After incubation, the residual amount of the test compound was analyzed and compared with that obtained from the absorption experiment in another group of rats. Visible absorption spectra were also measured before and after the above experiments with a Hitachi EPS-3T recording spectrophotometer (Hitachi Co., Ltd., Tokyo) to check for metabolic change of the test compounds.

Measurement of Absolute Viscosity——Kinematic viscosities of oily solvents were determined with an Ubbelohde viscometer (Kaburagi Kagaku Kikai Kogyo Co., Ltd., Japan) and their densities were measured by using a volume scale-corrected Cassia flask. These determinations were performed at 37°, and the absolute viscosity was calculated from the kinematic viscosity and density.

Determination of the Apparent Distribution Coefficient——Ten milliliters of 0.9% (w/v) NaCl aqueous solution (saline) was added to 4 ml of an oily solution containing the model compound (concentration: for PAAB, PHAB and OAAT, 5—40 mg/ml; for TS, 2.33—4.65 mg/ml; for epitiostanol, 2—5 mg/ml) and shaken well at 37° with a mechanical shaker until distribution equilibrium was reached. Our preliminary experiment had demonstrated that 2 hr was needed for this equilibrium, and that the volume change of each phase after equilibrium was small enough to neglect. After equilibrium had been reached, the unstable emulsion was centrifuged at 4000 rpm for 10 min to separate the transparent aqueous phase, part of which was withdrawn for analysis. The concentration of the model drug in the oily phase was calculated from its concentration determined for the aqueous phase and its initial concentration in the oily phase. The apparent distribution coefficient (K) was represented as the concentration ratio between the two phases. The value of K as determined above depended little on the concentration and was similar to the solubility ratio at 37° between the oil and saline solvents. For PANA, whose concentration in saline was too low to be determined accurately, the solubility ratio between the two solvents was used as the apparent distribution coefficient.

Analytical Method——(i) Samples from Animal Experiments: Sample ethyl acetate solutions containing azo compounds were adequately diluted with ethyl acetate and their optical densities were measured with a Hitachi UV-VIS spectrophotometer at 384, 348, 386, 450 and 510 nm for PAAB, PHAB, OAAT, PANA and Sudan III, respectively. Epitiostanol was determined by GLC after being transformed to olefin (5α-androst-en-17β-ol) in the following manner.[6] Five milliliters of sample solution (epitiostanol content, 0—160 μg) was shaken with 1 g of Al_2O_3 (active, neutral, I, E. Merck, Darmstadt) for 10 min and centrifuged at 3000 rpm for 5 min (this procedure was done to minimize interfering materials in the following GLC assay). To 1.5 ml of the supernatant was added 0.5 ml of internal standard solution (3β-acetoxy-5α-androstan in ethyl acetate, 0.25 mg/ml), then the mixture was evaporated to dryness in a Vapour Mix instrument (Tokyo Rikakikai Co., Ltd., Tokyo). The residue was dissolved in 5 ml of benzene and refluxed with about 30 mg of spongy active copper (Cu–Zn) for 40 min. The reaction mixture was filtered through Toyo filter paper No. 131, and 2 ml of the filtrate was evaporated to dryness. The residue was redissolved in about 200 μl of ethyl acetate and 1—2 μl of this was analyzed with a gas chromatograph, model GLC-4APTF, equipped with a flame ionization detector (Shimadzu Seisakusho, Ltd., Kyoto) under the following conditions. The column was a 1.5 m×5 mm glass tube packed with 3% SE-30 on Gas Chrom Q (80—100 mesh); carrier gas, 99.999% N_2 (flow rate, 60 ml/min); column, injection port and detector temperatures, 250, 268 and 288°, respectively. In the case of TS, 3 ml of sample solution was shaken with 0.6 g of an Al_2O_3-silica gel[7] mixture (1 : 1, w/w) for 10 min then centrifuged at 3000 rpm for 5 min (this procedure was performed for the same reason as in the case of epitiostanol). To 1 ml of the supernatant was added 100 μl of internal standard solution (3β-acetoxy-5α-androstan in ethyl acetate, 0.5 mg/ml). Two microliters of this mixture was used for GLC analysis as described above (column, injection port and detector temperatures were modified to 260, 280 and 293°, respectively).

(ii) Samples from Other Experiments: (1) Test Compounds in Oily Solutions: Sample Solutions for azo compounds were analyzed colorimetrically after dilution with chloroform (PAAB, 372 nm; PHAB, 345 nm; OAAT, 376 nm; PANA, 436 nm; Sudan III, 520 nm). Epitiostanol and TS were assayed by GLC as described above after dilution with ethyl acetate. (2) Test Compounds in Saline: Sample solutions of azo compounds and TS were analyzed spectrophotometrically as follows: PAAB and PHAB at 376 and 348 nm, respectively, after dilution with distilled water; OAAT and PANA at 379 and 470 nm, respectively, after 10/9-fold dilution with EtOH; TS at 241 nm after 10-fold dilution with EtOH. Epitiostanol was assayed by the GLC method described above after extraction with ethyl acetate.

Results

Comparison of Clearance of Model Compounds in the Injection Site between *in Vitro* Incubation and *in Vivo* Absorption Experiments

To date various experimental procedures have been proposed and used to evaluate the absorption rate of drugs parenterally administered: (1) observation of changes in the pharmacological effect with time; (2) following the blood level and/or urinary excretion, and pharmacokinetic analysis of the data; (3) following drug clearance at the injection site (local clearance method). We adopted the local clearance method since a sample oily solution injected intramuscularly remains localized, allowing almost complete recovery of the remaining drug. Apparent elimination of the drug observed by the local clearance method sometimes results not only from its transport to the blood or lymph system but also from its metabolic change at the injection site. Hence, in order to check the contribution of the latter factor in the case of the model compounds, an *in vitro* incubation experiment with removed muscle tissues was undertaken and the resulting data of % recovery for a given period were compared with those from the *in vivo* absorption experiment. Table I gives this comparison for each compound in the SO solution. For all compounds, the recovery was near 100% for the *in vitro* incubation experiment but considerably lower for the *in vivo* absorption experiment. Further, no significant change of the visible absorption spectrum was observed before and after these two experiments. These results showed that the metabolic change of these model compounds in the muscle was almost negligible and their apparent clearances observed here resulted mainly from transport into vascular systems. These findings support the view that the model compounds and the absorption experiment procedure adopted here are appropriate for analyzing the true absorption phenomena of practically water-insoluble drugs injected intramuscularly.

Table I. Comparison of % Recovery between *in Vitro* Incubation with Removed Muscle Tissues and *in Vivo* Absorption Experiment[a]

Compound	Time (hr)	% recovery[b]	
		In vitro	*In vivo*
PHAB	2	97.0 (2.9)	38.1 (3.7)
PAAB	2	98.9 (5.8)	52.9 (5.2)
OAAT	6	99.6 (4.3)	65.0 (8.5)
PANA	6	100.0 (5.7)	91.3 (4.2)
Sudan III	6	100.5 (0.9)	98.8 (0.8)

a) Vehicle, SO; initial concentration (C_0), 0.5 mg/ml; injection volume (V_0), 0.05 ml.
b) Each value represents the mean of 3 experiments with the standard deviation in parentheses.

Clearance of Sesame Oil from the Injection Site

Various vegetable oils such as sesame oil, olive oil, cotton seed oil, peanut oil and castor oil are usually used as oily solvents for preparations. Sesame oil (SO) is most commonly used in Japan. Since these oils consist not of a single component but of various triglycerides such as olein, linolein, stearin, palmitin and myristin, and other natural products, direct evaluation of their absorption characteristics from the injection site seems impossible. Recently, some reports have appeared on intramuscular absorption of mineral oil,[8] ^{14}C-methyl oleate,[5] and ^{14}C-tripalmitin mixed with sesame oil.[9] All these investigations indicated that these oils are absorbed very slowly.

An oil vehicle may be eliminated from the injection site mainly *via* one or both of two routes: (a) absorption after being dissolved in the body fluids or transformed into some hydrophilic compounds and (b) direct absorption after division into micro-droplets. Table I shows that Sudan III, which could scarcely be released from the SO vehicle into the aqueous phase,

was insignificantly absorbed for at least 6 hr after injection. Thus, with Sudan III as a tracing material, the absorption of SO was examined by an indirect method, as described in "Experimental." The results appear in Table II. The residual volume (V) of SO and the residual amount (W) of Sudan III 6 hr after injection had little changed from their initial values, but subsequently decreased gradually. This observation showed that SO was scarcely absorbed during the first several hours after injection, but that after this it might be very slowly absorbed, presumably *via* both routes mentioned above.

Table II. Absorption of Sesame Oil Estimated by the Indirect Method

Time (hr)	W (μg)[a]	C (μg/μl)[b]	V (μl)[c]
0	272.0 (2.1)	5.07 (0.01)	53.6 (0.4)
6	268.7 (1.0)	5.03 (0.02)	53.5 (0.2)
20	242.9 (7.3)	5.00 (0.13)	48.6 (1.9)
41	239.4 (24.3)	5.26 (0.28)	45.5 (5.2)

a) Residual amount of Sudan III.
b) Sudan III concentration in the oily depot phase.
c) Calculated residual volume of SO. $V=W/C$. Each result is given as the mean of at least 3 experiments followed by the standard deviation in parentheses.

Time Course of Drug Absorption and Effect of Initial Drug Concentration

Fig. 1 shows a time course of intramuscular absorption of PAAB after injection of its SO solution. The initial concentration (C_0) and injection volume (V_0) are given in the legend. The upper figure (A) shows the value of the % remaining on a linear scale while the lower one (B) uses a logarithmic scale. From the linearity shown in Fig. 1B, this compound was expected to be absorbed mono-exponentially, that is, according to a 1st order rate process. In order to confirm this, the effect of the initial concentration (C_0) of PAAB in the SO solution on absorption was examined. Fig. 2 shows a comparison of time courses of PAAB absorption on a semilogarithmic scale from three solutions of different C_0. All these absorption profiles gave

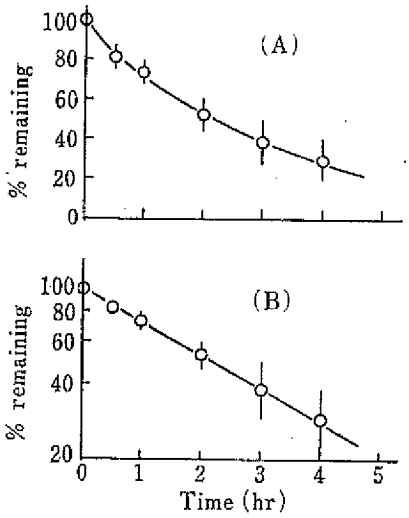

Fig. 1. Linear (A) and Semilogarithmic (B) Plots of % Remaining PAAB at the Injection Site *versus* Time (SO)

Each point represents the mean of 4 experiments. The vertical bar about the mean shows the standard deviation. C_0, 5 mg/ml; V_0, 0.05 ml.

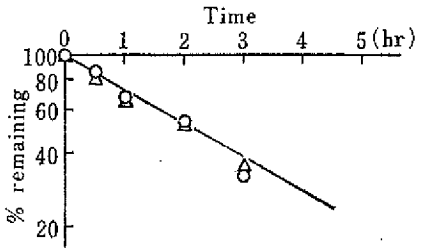

Fig. 2. Effect of Initial Concentration on PAAB Absorption following Intramuscular Injection (SO)

Key: ○, 0.5 mg/ml; △, 40 mg/ml; solid line, 5 mg/ml (shown in Fig. 1B). Each point represents the mean of at least 3 experiments. V_0: 0.05 ml.

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