phasise prodrugs which improve the pharmacokinetic properties of anticancer agents and, in particular, prodrugs which are activated selectively in tumour cells to the active drug.

2. Prodrugs with altered chemical stability, solubility or better tissue penetration

The chemical instability of some anticancer agents when dissolved in physiological saline is well recognised and is overcome, for example, in the case of reactive alkylating agents by ensuring rapid dissolution and injection, and, in the case of formulations of dimethyltriazenoimidazole carboxamide that are light unstable, by the use of dark vessels and the avoidance of sunlight. Other agents may decompose less slowly, but nevertheless this may be a problem when they are used as infusions over long periods of time. This problem can usually be overcome by a study of the mechanism of decomposition, followed by appropriate prodrug design to increase stability. Azacytidine, for example, is used in the treatment of acute myeloid leukaemia. When given by an intravenous bolus it causes severe and dose-limiting gastrointestinal toxicity. However, if the drug is slowly infused over a 5-day period this toxicity is eliminated, but such infusions are not really practicable because of the instability of azacytidine in aqueous solutions. Azacytidine is hydrolysed reversibly to the ring-opened formyl derivative, which is then converted slowly and irreversibly to the guanylurea (Fig. 1). While both the formyl and the urea derivatives have lower toxicity, they do not have antitumour activity so that over the long infusion period a gradually reducing concentration of azacytidine is being infused,

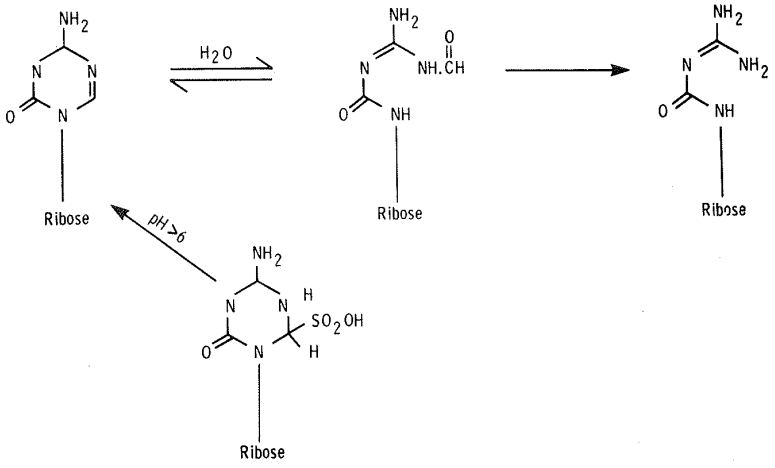


Fig. 1

hydrophilic nitroimidazoles concentrate less in neural tissues and are excreted more rapidly, both properties reducing neurotoxicity. These nitroimidazoles may also be suitable for oral administration, but since decreased lipophilicity will decrease oral absorption a prodrug may be required to obtain optimal activity [9]. A nitroimidazole has been synthesised which has a very low octanol/water partition coefficient and might be expected to be less neurotoxic than earlier radiation sensitisers such as misonidazole, which has dose-limiting neurotoxicity. In order to increase absorption from the gut the prodrug acetyl ester was synthesised and shown to be converted completely to the drug in the first pass through the liver (Fig. 3).

In some cases it is necessary to reduce the water solubility of a drug by the synthesis of a more fat-soluble prodrug. Anticancer agents such as 6-mercaptopurine are also useful in the treatment of psoriasis, but, because they do not penetrate the skin, must be given systemically, with resulting toxicity. A soft alkylated derivative of 6-mercaptopurine may be used as a topical treatment of psoriasis [10] since it is transported effectively through epidermal barriers and is then converted, firstly by esterases and then spontaneously to the active thiopurine (Fig. 4).

3. Prodrugs which overcome acquired resistance

Often if the mechanism by which a cell acquires resistance to a drug is understood it is possible to design a prodrug to overcome this resistance. Thus, 6-mercaptopurine, like many antipurines and antipyrimidines, requires intracellular conversion to a nucleotide before it exerts its inhibitory action. 6-Mercaptopurine is converted by hypoxanthine, guanine phosphoribosyl transferase (HGPRT) to the nucleoside monophosphate (Fig. 5), which interferes with cell growth by a number of

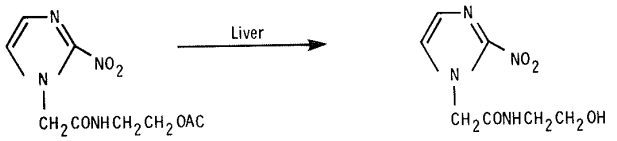


Fig. 3

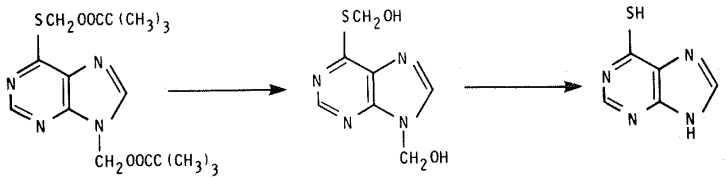


Fig. 4

4. Prodrugs with improved pharmacokinetic properties

Most anticancer drugs are cell cycle-specific, meaning that they are particularly toxic to cells in cycle. Some drugs are even more specific, being cytotoxic to cells only in a particular phase of the cell cycle. For these agents the correct pharmacokinetic profile is essential if selective inhibition of tumour cells is to be obtained. Cytosine arabinoside (Ara-C), for example, acts after its intracellular conversion to the triphosphate, in which form it is an inhibitor of DNA polymerase. Therefore, it is S phase-specific and to achieve its optimal anti-tumour effect it must be present in the tumour environment at a toxic concentration for the period of time that it takes all the cells of the asynchronously growing tumour to enter and pass through the S phase. However, Ara-C as well as being activated to its triphosphate is also a substrate for cytidine deaminase and is converted by this enzyme to an inactive product (Fig. 7). This would result in a rapid clearance of Ara-C from the body and its profile after intraperitoneal or intravenous injection would be as indicated diagrammatically in Figure 8. After either route of administration the phar-

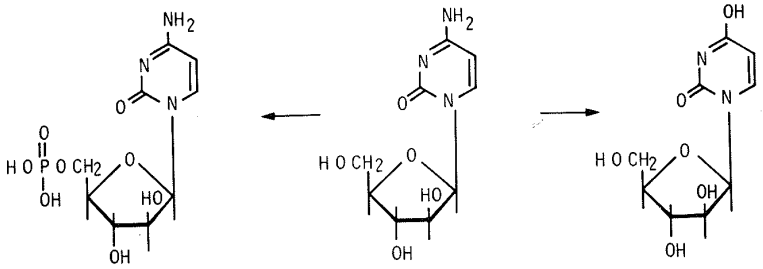


Fig. 7

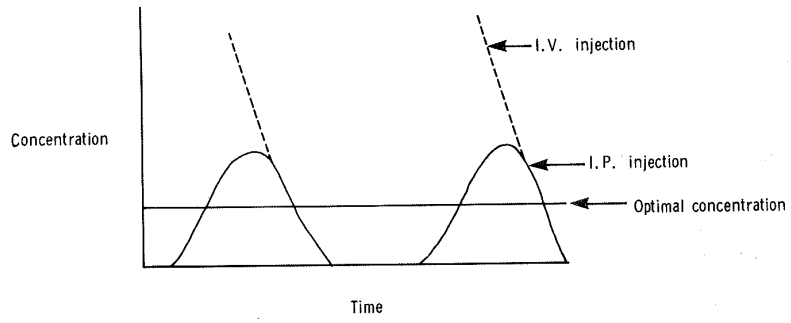


Fig. 8

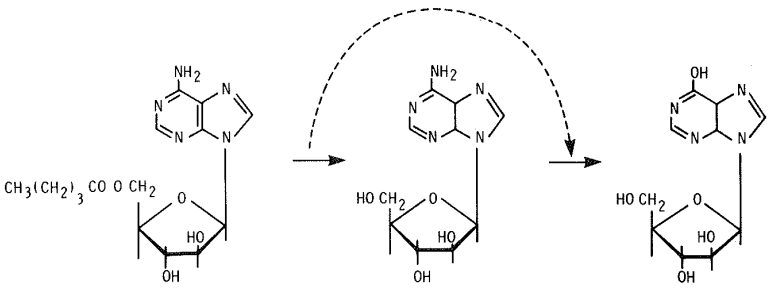


Fig. 10

from steroids such as cortisone, prednisolone and a range of anti-inflammatory steroids [24]. Some but not all were superior to Ara-C alone in tumour-bearing animals, and it is of interest that prodrug activation appeared to take place inside the cells rather than in the blood. Clearly, this could form the basis for the design of prodrugs with activity against cells, with acquired resistance to Ara-C as a result of loss of the activating enzyme. Some prodrugs of Ara-C, such as Ara-CTP-L-dipalmitine, are not substrates for cytidine deaminase and are very effective against tumour cells in culture [25]. They might be readily taken up by cells and then degraded to Ara-CTP. In such a situation cells resistant to Ara-C because of loss of the kinase would remain sensitive to this type of prodrug. In fact, diacylglycerol derivatives of cystosine arabinoside do have an effect against some mouse tumours which have acquired resistance to Ara-C [26]. Other analogues of Ara-C which have been studied include phospholipids and polyglutamates and an N^4-palmitoyl derivative which was effective when given orally to tumour-bearing mice [25 – 30].

Following similar lines esters, glutamyl conjugates, amides, hydrazides and hydroxamic derivatives have been made of methotrexate [31 – 33]. Although in many cases the rationale has been to produce slow-release prodrugs forms of methotrexate, others have been made which may be taken up readily by cells by passive diffusion, and thus be active in cells resistant to methotrexate in which the active transport mechanism has been lost. In the course of these studies some interesting findings have been made. Most importantly, diesters of methrotrexate may be cytotoxic to leukaemia cells in their own right, an effect which is only partially reversed by folinic acid, indicating that they may be acting by more than one pathway. Furthermore, in mouse serum there is a regioselective hydrolysis of the diester, the α-carboxyl ester being hydrolysed much more readily than the γ [31]. Studies on esterases as prodrugs have emphasised that the rodent may not be a satisfactory model for humans since serum esterases in the former are much more active than in humans.

N,N-Dibenzyldaunorubicin has no activity against tumour cells in vitro but is active in vivo. suggesting that in whole animals it acts as a prodrug, with possible dif-

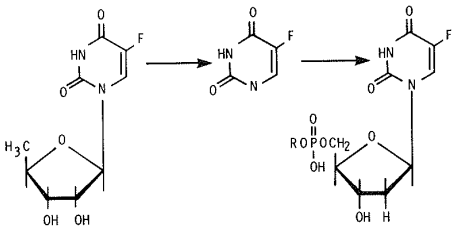


Fig. 12

and cytotoxicity. Highly reactive nitrogen mustards are likely to be very toxic (both to tumour cells and to normal tissues) and poorly reactive ones very much less toxic. Figure 13 shows that as the alkylating activity varies over a 25-fold range, the LD_{50} varies from 40 to 3000 μmol/kg. Very small changes in structure, e.g., *p*-hydroxylation, can greatly alter reactivity, and hence toxicity [40]. On this basis prodrugs can be designed which are poor alkylating agents and non-toxic, but which act as substrates for enzymes which transform them to highly reactive and cytotoxic agents. Clearly, for there to be selective tumour cell kill, the prodrug must be a good substrate for the enzyme under physiological conditions and the enzyme ideally should be present uniquely in the tumour cells, or at least at much higher levels in the tumour than in normal tissues, especially those which are also sensitive to this type of agent. A further prerequisite is that once formed from the prodrug, the drug

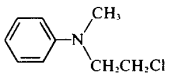
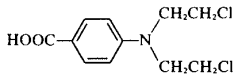
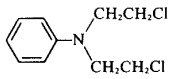
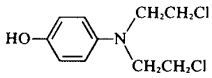
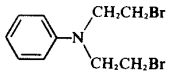
Compound	Alkylating activity ($K_{50} \times 10^3$)	Toxicity (LD_{50}, μmole/kg)
	4·9	3000
	4·6	915
	13·0	367
	48·6	74
	123·0	39

Fig. 13

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