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INCREASED SENSITIVITY OF THE WALKER TUMOUR TOWARDS AROMATIC NITROGEN MUSTARDS CARRYING BASIC SIDE CHAINS FOLLOWING GLUCOSE PRETREATMENT

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Abstract—Neoplastic tissue, which is more acidic than normal tissue, should concentrate basic drugs. The pH difference can be accentuated by glucose pretreatment and it has been shown that the anti-tumour activity of some aromatic nitrogen mustards carrying sidechains containing basic groups with suitable pK_a values is enhanced by glucose treatment. Certain epoxides should be even more suitable for exploiting this tissue difference. Thiols should selectively protect normal cells against alkylating agents as a consequence of the higher concentration of the nucleophilic anionic form therein.

NUMEROUS alkylating agents such as sulphur and nitrogen mustards, epoxides, ethyleneimine derivatives, and methanesulphonates are toxic towards dividing cells. The growth of cancer cells is inhibited but other cells in normal tissue, such as those in the gastric mucosa, gonadal tissue and haematopoietic tissue, are also affected. One has sought for some characteristic property of the cancer cell which might be exploited to obtain a localization of the cytotoxic effect.

A feature of neoplastic tissue is its lower pH relative to normal tissues which is consequent on the relative accumulation of lactic acid formed by glycolysis. This property can be enhanced by glucose administration. Thus Voegtlin *et al.*[1] showed that following glucose administration the pH of tumour tissue may fall from 6·9 to 6·3. Kahler and Robertson[2] found that normal liver tissue had pH 7·4 and hepatoma had pH 7·0 which could be reduced to 6·4 by glucose treatment—the pH of normal tissue being unaffected. Stevens *et al.*[3] showed that sulphapyrazine, which is less soluble under acid conditions, was selectively deposited in the tumour area of glucose-treated rats which carried a Walker carcinoma. Although the pH as measured by microelectrodes is in most cases that of extracellular fluid it seems not unreasonable to assume that the lactic-acid-producing cells are at an even lower pH. This would not affect the argument which follows except in so far as the pK_a requirement for maximum activity in basic derivatives may be displaced to lower values.

Basic drugs will concentrate in cells with lower internal pH as a consequence of the greater ability of the non-ionized form to diffuse through cellular membranes.[4] The relative concentration of a base in region p (plasma) compared with region c (cell) from which it is separated by a lipoid barrier is given by:

$$\frac{\text{concentration of base in } c}{\text{concentration of base in } p} = \frac{1 + 10\,(pK_a - pH_c)}{1 + 10\,(pK_a - pH_p)}$$

Fig. 1 shows the values of this ratio (R) for bases of various pK_a values assuming that the pH of plasma is 7·4, of normal cells is 7·0, and of cancer cells is 6·0—a value which may be reached by suitable glucose pretreatment. Under these conditions a base of pK_a greater than 8 can achieve ten times the concentration in cancer cells as in normal cells (Fig. 2, curve 1). Since strong bases will be present mainly in the non-diffusing

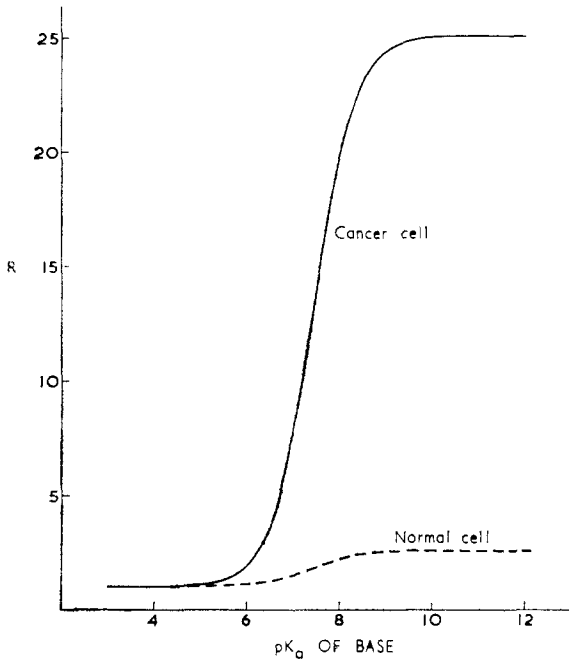


FIG. 1. Variation of R = concentration of base in cell/concentration of base in plasma with pK_a of base.

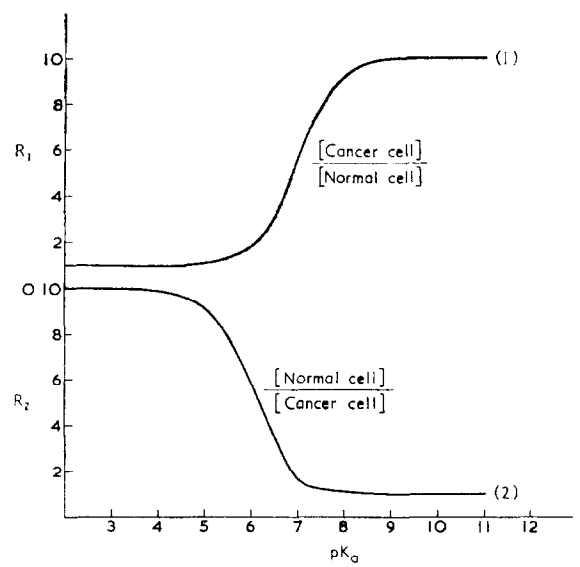


FIG. 2. (1) Ratio R_1 of concentration in cancer cell to concentration in normal cells for bases. (2) Ratio R_2 of concentration in normal cell to concentration in cancer cell for acids.

ionic form under the conditions being considered, the optimum pK_a is that at the shoulder of curve 1 in Fig. 2. Thus bases with a pK_a between 8 and 9 should be most suitable for concentration within cancer cells. Even if a pH as low as 6 cannot be achieved some concentration of the base in the neoplastic cell can be attained and Fig. 3 shows how the relative concentration changes with pH.

The drug concentration is a sum of the concentration of the dissociated and undissociated forms, the concentration of the undissociated form being the same on both sides of the lipoid barrier. If the charged form—the protonated form in the case of a basic drug—is biologically inactive then no advantage would be gained but if both forms are equally active then a greater effect will be manifest in the more acid cell.

Some preliminary results using aromatic nitrogen mustards which should be active over the pH range being considered are now reported. In the first experiment (Table 1) the basic compound, N-(*p*-di-(2-chloroethyl)aminophenethyl) methylamine, CB 3039 (pK_a 8·9, Ref. 5), was administered to animals on the day following the implantation of a Walker tumour as a single intraperitoneal injection in arachis oil. A parallel

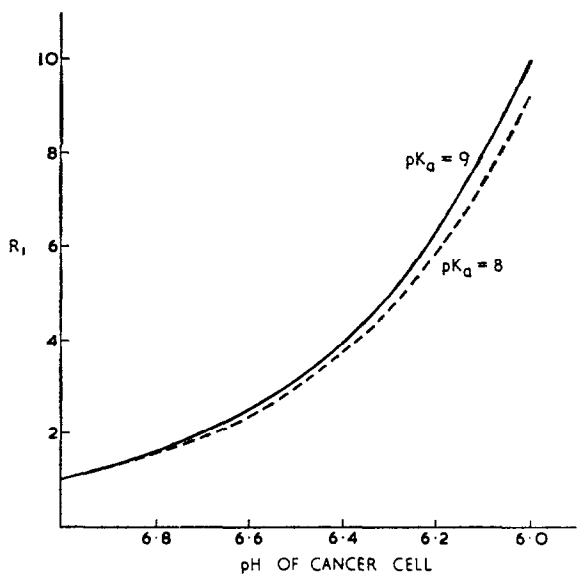


FIG. 3. Ratio (R_1) of concentration in cancer cell to concentration in normal cell for different pH values in cancer cell for base of pK_a 8 and 8 and 9 (normal cell pH = 7).

experiment was carried out in which three doses of 7·5 g/kg of glucose were administered (i.p. in water) 1 hr before the drug, with the drug, and 1 hr after the drug. Table 1 shows that the total weight of tumours of the control group divided by that of the treated group (C/T ratio) was increased by glucose treatment. This enhancement of activity was confirmed in a second experiment in which five doses of glucose were administered (Table 2). A similar increase in the tumour growth inhibitory effect following glucose injection was also demonstrated in the case of the ester of melphalan (CB 3177) (Table 3). Injection of glucose alone does not inhibit tumour growth—a slight increase in growth rate was, in fact observed (Table 4). Neutral (CB 1074,) acidic (CB 1348, chlorambucil), and zwitterionic (CB 3025, melphalan) aromatic nitrogen mustards would not be expected to concentrate selectively within the more

acidic cancer cells and as expected their activity was not enhanced by glucose treatment (Table 4). In fact, CB 1348 should be less concentrated in acidic cells and there is an indication of a reduction in anti-tumour activity following glucose treatment in this case. The pK_a of the aromatic chloroethylamino group present in all the mustards is low, about 2, and need not be considered in the present context.

TABLE 1. TUMOUR WEIGHTS

Controls	CB 3039 (8 mg/kg)	CB 3039 (8 mg/kg) plus glucose (7·5 g/kg at −1, 0 and +1 hr)
72	46	10
66	40	9
64	20	2
58	20	nil
44	10	nil
33	nil	nil
Total 337	136	21
C/T	2·5	16

TABLE 2. TUMOUR WEIGHTS

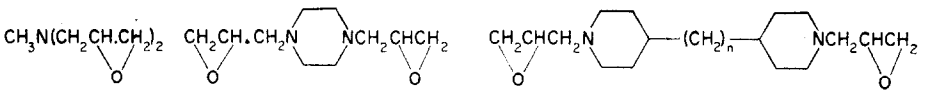
Controls	CB 3039 (8 mg/kg)	CB 3039 (8 mg/kg) plus glucose (5 g/kg at −2, −1, 0, +1, and +2 hr)
52	20	nil
52	3	nil
51	3	nil
36	2	nil
33	nil	nil
5	nil	nil
Total 229	28	nil
C/T	8·1	∞

TABLE 3. TUMOUR WEIGHTS

Controls	CB 3177 (0·5 mg/kg)	CB 3177 (0·5 mg/kg) plus glucose (5 mg/kg at −1, 0, and +1 hr)
61	25	13
46	23	nil
40	11	nil
35	1	nil
31	nil	nil
24	nil	nil
Total 237	60	13
C/T	4	18·2

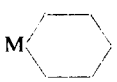
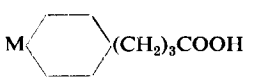
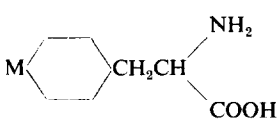
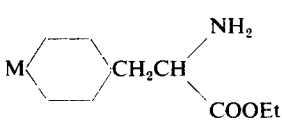
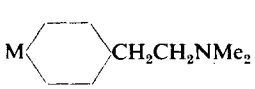
It is of some interest to note that peptide derivatives of 1-amino*cyclo*pentanecarboxylic acid[6] and of melphalan[7] which contain a free amino group are more active tumour inhibitors than those in which this group is acylated.

Greatest advantage will be obtained by selective concentration of basic drugs if the cationic form of the drug is more active than the uncharged form. Certain epoxides and ethyleneimine derivatives are chemically more reactive in acid solutions and would be expected to export a greater toxic action on cancer cells.[8, 9] Basic epoxides of appropriate pK_a values (8–9, see above) would be well suited to selective concentration and enhanced reaction in the more acidic cells. A number of compounds of this type have been synthesized by Gerzon *et al.*,[10] for example (I), (II), and (III):



(I) (II) (III)

TABLE 4

Compound	Dose (mg/kg)	*C/T* ratio	
		Alone	With glucose
CB 1074  (Ref. 12)	20	3·7	3·5*
CB 1348  (Ref. 13)	1·5 1·0	7·2 1·6	4·5* 1·0*
CB 3025  (Ref. 14)	0·5	7·5	7·5*
CB 3177 	0·5	4	18·2*
CB 3039  (Ref. 5)	8 8	8·1 2·5	∞† 16‡
Glucose	*	0·5 0·75	

M = $-N(CH_2CH_2Cl)_2$

* 5 g/kg of glucose at −1, 0 and 1 hr after drug.

† 5 g/kg of glucose at −2, −1, 0, 1 and 2 hr after drug.

‡ 7·5 g/kg glucose at −1, 0 and 1 hr after drug.

Compound (I) has pK_a values of < 3 for the two nitrogen atoms, the values for (II) are < 3 and 6·1, for (III, $n = 0$) are 6·8 and 7·9, and for (III, $n = 1$) are 7·0 and 8·0. The relative biological effectiveness of the compounds against a mouse leukemia are (I) 0·1, (II) 1, (III, $n = 0$) 5–10, (III, $n = 1$) 10–20 and these results are broadly as expected from the above considerations. Creech *et al.*[11] showed that (III, $n = 0$) had a chemotherapeutic index superior to that of (II) when tested against a mouse ascites tumour.

The use of epoxides, especially any with pK_a values between 8 and 9, coupled with prior glucose administration should lead to enhanced action on cancer cells. This approach to the problem of obtaining selectivity of action is being actively pursued in this Institute. If this technique is employed it will probably be necessary to reduce stomach acidity, e.g. by bicarbonate administration, in order to protect the gastric mucosa during the time that the alkylating agent is able to interact with tissues.

Thiols, especially in the more nucleophilic RS$^-$ form, react readily with alkylating agents and can reduce toxicity by "mopping up" the reactive drug. Since they are weak acids they will, in contrast with bases, concentrate in less acidic regions. Thus a higher concentration of the thiol will be present in normal cells as compared with cancer cells and of this greater amount a greater proportion will be in the reactive RS$^-$ form. As a consequence normal cells will be selectivity protected against the action of alkylating agents of all types by prior administration of thiols. Simple thiols or weakly acidic derivatives, such as mercaptocarboxylic acids, could be used but basic thiols, such as cysteamine, or thiols with active transport, such as cysteine would seem to be less useful for selective protection. Fig. 2 which indicates R_2 values for acids shows that for optimum selective concentration in normal cells under the conditions chosen the pK_a of the thiol acid should be about 4 to 5. Experiments are in progress in this Institute to examine the protective action of acidic thiol derivatives.

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