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THE EFFECT OF ANTI-CANCER DRUGS ON THE PLASMA DISPOSITION OF ANTIPYRINE AND THE BILIARY EXCRETION OF PHENOLPHTHALEIN IN THE RAT

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Abstract—The uptake and plasma disposition of antipyrine in the rat have been examined as a possible model for estimating the metabolic effects caused by the administration of the cytotoxic anti-cancer drugs to man. Pretreatment of the rats with the anti-cancer drugs retarded and decreased absorption from the stomach and the elimination of antipyrine from the plasma was also considerably decreased. In parallel with these findings the marked reeduction in the biliary excretion of phenolphthalein indicated a decrease in hepatic function caused by the cytotoxic drugs. *In vitro* investigations appeared to suggest the most likely cause of the prolonged antipyrine plasma half-life was the reduction in hepatic cytochrome P450 content.

The pharmacokinetics of antipyrine in man are being increasingly investigated as a diagnostic aid to evaluate liver function and also as an indicator of the probable disposition of other administered drugs [1, 2]. The value of antipyrine (and other drugs) pharmacokinetic studies in the prediction of the likely disposition of drugs in liver disease has recently been reviewed [3]. In this study the uptake and elimination of antipyrine into and from plasma following oral administration has been investigated in rats which have been pretreated with one of the more commonly used anti-cancer drugs to estimate their effect on gastrointestinal absorption and hepatic metabolism. The cytotoxic drugs administered were at a level comparable with the therapeutic dose in man. The drugs selected were an example of each of the main types of anti-cancer drugs; alkylating agents—cyclophosphamide; antimetabolites—methotrexate and 5-fluorouracil; plant alkaloids—vincristine; antibiotics—actinomycin-D. Phenolphthalein which is excreted almost exclusively in the bile was administered to similarly pre-treated rats to indicate whether any delay in excretion was due to hepatic or renal damage [4]. The hepatic microsomal enzymes responsible for the metabolism of antipyrine and phenolphthalein were also examined *in vitro*.

A high correlation has beeen reported between antipyrine and aryl hydrocarbon hydroxylases, indeed results using benzo(a)pyrene as substrate suggests that the same monooxygenase enzyme system metabolizes benzo(a)pyrene and antipyrine [5, 6]. The level of benzo(a)pyrene hydroxylase *in vitro* was thus used as an indication of antipyrine hydroxylase activity *in vitro*.

MATERIALS AND METHODS

Chemicals. Antipyrine, actinomycin D, benzo(a)-pyrene, phenolphthalein and 5-fluorouracil were purchased from Sigma, Kingston-upon-Thames, U.K. Vincristine sulphate (Oncovin) from Eli-Lilley, Basingstoke, U.K., cyclophosphamide (monohydrate) from Koch-Light, Colnbrook, U.K., sodium methotrexate from Cyanamid, Pearl River, NY, U.S.A. G[^{3}H] Benzo(a)pyrene, specific activity 10 Ci/m-mole was supplied by the Radiochemical Centre, Amersham. All other reagents used were of 'analar' grade and obtained from Hopkin and Williams, Chadwell-Heath, Essex.

Animals and treatment. Male Sprague–Dawley rats of body wt 300–400 g were purchased from Olac, Birmingham. The animals were given either an oral dose of methotrexate (1 mg/animal) or cyclophosphamide (4 mg/kg) in saline (2 ml/kg) once daily for 5 successive days, or an i.v. injection of vincristine (0.03 mg/kg) or actinomycin D (0.1 mg/kg) in saline (1 ml/kg) once weekly for 3 successive weeks, or an i.p. dose of 5-fluorouracil (12.5 mg/kg) in saline (2 ml/kg). Control animals received an oral dose of saline (2 ml/kg) once daily for 5 successive days. Two days after cessation of treatment the animals received, after an overnight fast, an oral dose of antipyrine (18 mg/kg) in saline (2 ml/kg). Blood (10 ml) was removed by cardiac puncture from groups of three rats before and 5, 10, 15, 30, 60 min, 2, 4, 8, 12, and 24 hr after dosing. The level of antipyrine present in the plasma was determined by the method of Brodie *et al.* [7].

Methotrexate was also administered to a further six animals as a single dose (5 mg/animal) in saline (2 ml/kg) and these animals were cannulated as described.

Biliary cannulations. Two days after completion of dosing with the anti-cancer drugs groups of six animals, representative of each type of drug pretreatment and six control rats, had a cannula inserted into the median bile duct essentially as described by Abou-El-Makarem *et al.* [8] except that light ether anaesthesia was used. The animals were transferred to restraining cages and allowed to recover from the anaesthetic before phenolphthalein (10 mg/kg, in a 20% ethanol: 80% polyethylene glycol solution) was introduced, by injection via a tail vein. Bile was

Table 1. The levels of antipyrine in the plasma of pretreated and control rats at intervals of time after the oral administration of the drug (18 mg/kg)

Time	Plasma level of antipyrine* in animals pre-treated with: Saline (control)	Metho-trexate	Cyclo-phosphamide	Vincristine	5-Fluoro-uracil	Actino-mycin-D
5 min	12.4 ± 0.89	5.0 ± 0.50	3.5 ± 0.65	6.35 ± 0.40	9.5 ± 0.45	12.1 ± 0.66
10 min	20.44 ± 1.48	7.39 ± 1.16	10.4 ± 1.20	6.87 ± 0.60	9.2 ± 0.73	15.5 ± 0.89
15 min	21.16 ± 1.35	7.52 ± 0.47	10.8 ± 0.86	7.7 ± 0.62	8.5 ± 0.85	16.2 ± 0.90
30 min	4.85 ± 1.27	8.56 ± 0.78	14.7 ± 1.02	10.14 ± 0.65	10.5 ± 0.53	21.7 ± 0.46
60 min	4.13 ± 1.17	8.84 ± 0.74	8.9 ± 0.72	9.47 ± 0.57	9.1 ± 0.61	23.9 ± 0.70
2 hr	5.84 ± 0.95	6.77 ± 0.55	8.5 ± 0.92	11.01 ± 1.07	3.2 ± 0.68	18.3 ± 0.52
4 hr	1.74 ± 0.41	5.50 ± 0.64	6.0 ± 0.85	10.62 ± 1.04	9.5 ± 0.86	11.9 ± 1.09
8 hr	0.80 ± 0.36	2.63 ± 0.43	5.7 ± 0.47	3.66 ± 0.42	7.4 ± 1.05	8.9 ± 0.65
12 hr	—	2.47 ± 0.27	3.7 ± 0.55	3.86 ± 0.30	6.7 ± 0.77	6.6 ± 0.65
24 hr	—	1.42 ± 0.52	3.7 ± 0.43	3.23 ± 0.41	6.7 ± 0.74	7.4 ± 0.74

* Expressed as μg equivalents of antipyrine/ml plasma.
— Not detected.
In all cases the value quoted is the mean, followed by the S.E.M., for duplicate analysis of the plasma taken from three animals.

collected hourly for a period of 6 hr after dosing and the excretion of phenolphthalein monitored as described by Millburn *et al.* [4].

Enzyme incubations. Six control animals and rats representative of each type of drug pretreatment having received neither antipyrine or phenolphthalein were killed by cervical dislocation 2 days after the completion of dosing with the cytotoxic drugs and their livers excised and chilled on ice. The hepatic tissue was dissected with steel scissors and homogenized in 4 vol. of cold 0.25 M sucrose containing 50 mM Tris–HCl buffer (pH 7.4) in a Potter–Elvehjem homogenizer (five passes). The homogenate was centrifuged at 10,000 *g* for 20 min and the resultant supernatant centrifuged at 100,000 *g* for a further 60 min. The final supernatant was discarded and the microsomal pellet washed in 1.15% KCl containing 0.05 M Tris–HCl buffer, recentrifuged and then resuspended. The protein content was determined by the method of Lowry [9] and the microsomal suspension diluted with further KCl buffer solution to give a final concentration of 3 mg protein/ml. Cytochrome P450 levels were determined by the method of Omura and Sato [10]. The level of benzo(a)pyrene hydroxylation was estimated by a radiometric technique [11] and UDP glucuronyl transferase activity was determined essentially as described by Isselbacher [12] except that phenolphthalein was used as a substrate for the enzyme.

Radiochemical analyses. The radiochemical content of duplicate samples was determined in a toluene, triton-based scintillant mixture, counting each sample for two cycles of 10 min duration. Counting efficiency was determined by internal standardisation.

RESULTS

The level of antipyrine in the plasma. The levels of antipyrine in the plasma of control and pre-treated rats following oral administration of the drug are given in Table 1, *and a comparison between methotrexate-pretreated rats and the controls following oral or i.v. administration in Fig. 1.* Following oral administration, the maximal plasma level of antipyrine is achieved within 15 min in the control animals, but in the pretreated animals the maximal plasma levels are achieved at times varying from 30 min to 2 hr. Thus pre-treatment with the anti-cancer drugs by various routes of administration significantly retards the uptake of antipyrine from the gastrointestinal tract. Except in the case of animals pretreated with actinomycin-D, the maximal plasma level of antipyrine realised after prior administration of any of the other cytotoxic drugs was appreciably less than that of the controls. The lowest maximal plasma level of antipyrine (42 per cent of the control) was observed in the case of animals pretreated orally with methotrexate but vincristine, administered i.v., also lowered absorption by a comparable amount (52 per cent of the control).

The levels of antipyrine in the plasma of the pretreated animals remain comparatively high even after intervals of time of 12 and 24 hr, at which

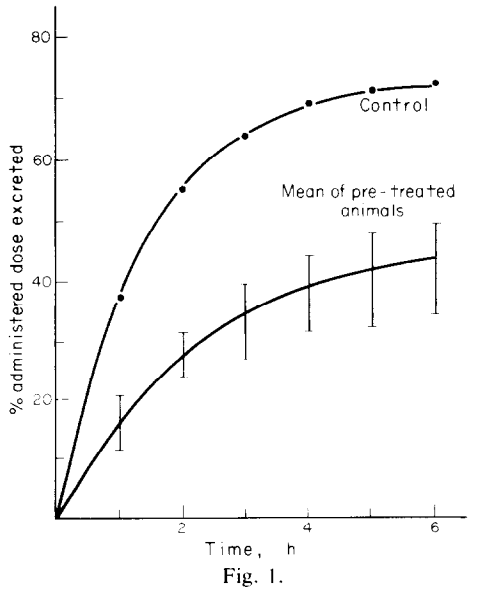

Fig. 1.

Table 2. The excretion of phenolphthalein* in the bile of control and rats pretreated with anti-cancer compounds following i.v. administration (10 mg/kg)

	Biliary excretion in animals pre-treated with:													
	Saline (control)		Methotrexate†		Methotrexate‡		Cyclophosphamide		Vincristine		5-Fluorouracil		Actinomycin-D	
Time (hr)	vol. (ml)	%	vol. (ml)	%	vol. (ml)	%	vol. (ml)	%	vol. (ml)	%	vol. (ml)	%	vol. (ml)	%
1	1.1	37.5 ± 0.89	1.0	20.5 ± 0.94	0.9	17.4 ± 0.55	1.1	14.7 ± 2.0	1.2	15.2 ± 2.05	0.6	13.6 ± 2.60	1.2	11.6 ± 0.47
2	1.3	18.0 ± 1.18	1.1	10.9 ± 1.54	1.2	11.9 ± 1.29	1.2	10.0 ± 0.52	1.4	16.4 ± 1.33	0.9	13.4 ± 1.11	1.2	12.5 ± 0.87
3	1.2	8.1 ± 0.72	1.1	7.9 ± 0.41	1.1	6.1 ± 0.27	1.1	4.0 ± 0.16	1.4	7.8 ± 1.11	1.0	7.3 ± 0.67	1.1	9.5 ± 0.65
4	1.1	5.4 ± 0.34	1.2	4.3 ± 0.31	1.0	3.5 ± 0.26	1.3	2.9 ± 0.11	1.4	4.9 ± 1.05	1.0	4.4 ± 0.36	1.1	6.1 ± 0.52
5	1.1	2.0 ± 0.32	1.2	3.1 ± 0.26	0.9	2.6 ± 0.16	1.1	1.6 ± 0.22	1.4	3.4 ± 0.13	0.9	2.9 ± 0.40	1.1	4.4 ± 0.49
6	1.1	1.5 ± 0.13	1.0	2.2 ± 0.11	1.0	1.0 ± 0.08	1.1	1.9 ± 0.22	1.3	1.8 ± 0.26	0.9	2.4 ± 0.33	1.0	3.9 ± 0.43
Total	6.9	72.5 ± 1.71	6.6	48.9 ± 1.09	6.1	42.5 ± 1.74	6.9	35.1 ± 1.36	8.1	49.7 ± 1.74	5.3	44.0 ± 2.82	6.7	48.0 ± 1.40

* The phenolphthalein excreted is expressed as a percentage of the administered dose voided in the total volume of bile collected per hr.
† Administered daily (1 mg/animal) for 5 successive days.
‡ Administered as a single dose (5 mg/animal).
In all cases the figures quoted are the mean values and the S.E.M. of the results obtained with six animals.

time the drug was not detected in the control animals. This effect is due to a delayed removal of antipyrine from the blood and the prolonged slow absorption from the gut.

Excretion of phenolphthalein in the bile. Figure 1 represents the effect of pretreatment with the anti-cancer drugs upon the cumulative excretion of phenolphthalein in the bile over 6 hr. The average volumes of bile and the percentage of the dose excreted is given in Table 2. Except in the case of 5-fluorouracil none of the anti-cancer drugs decreased the rate of bile production but the phenolphthalein excretion was reduced by a similar appreciable amount (a range of 23.6–37.4 per cent less of the administered dose than the controls in 6 hr) by each of the anti-cancer drugs. There was no significant difference in the reduction of phenolphthalein clearance in the bile between animals pretreated with a single oral dose of methotrexate (5 mg) and a similar dose given over 5 days (5 × 1 mg); 42.5 and 48.9 per cent of the dose in 6 hr respectively.

In vitro *assays.* From Table 3 it is seen that the effects of pretreatment with the anti-cancer drugs upon the various hepatic parameters investigated were not uniform. Thus all the drugs except methotrexate and 5-fluorouracil significantly increased the microsomal protein content of the liver whereas all the drugs lowered the cytochrome P450 content of the liver. The aryl hydrocarbon hydroxylase activity level appears to have increased except in the case of actinomycin D, and in the case of vincristine it was considerably lowered. UDPGA transferase activity appears to be unaffected by the pretreatment except in the case of actinomycin D when the level increased almost 3-fold although this drug is a known inhibitor of UDPGA transferase synthesis.

DISCUSSION

The effect of the anti-cancer drugs upon absorption from the gut appears to be similar to various forms of gastro-intestinal disease where there is a reduction in both rate and amount of absorption [13]. Such an effect would have been predictable in the case of orally-administered methotrexate but the decrease in antipyrine uptake caused by pretreatment with the other cytotoxic drugs by the i.v. and i.p. route is somewhat more surprising. Possibly these drugs reduce the replication of the cells of the gastrointestinal tract leading to an accumulation of keratinized, less-permeable cells. Many examples of the use of the plasma elimination of antipyrine to predict the hepatic clearance of other drugs under various conditions *in vivo* have been reported [3]. Such a comparison between the gastro-intestinal absorption of antipyrine and other drugs would be more difficult to establish.

However, antipyrine is rapidly and completely absorbed from the gut under normal conditions and presumably other less-well absorbed drugs would be

Table 3. The effect of pretreatment with anti-cancer drugs on the livers of rats*

Treatment	Average liver wt (g)	Microsomal protein (mg/g)	P450 (nm/mg protein)	Benzopyrene hydroxylase activity†	UDPGA transferase† activity
Control	10.0 ± 0.22	20.6 ± 0.43	1.12 ± 0.04	0.35 ± 0.068	5.4 ± 0.30
Methotrexate	15.8 ± 0.28	21.8 ± 0.30	0.92 ± 0.09	0.63 ± 0.055	5.1 ± 0.29
Cyclophosphamide	11.6 ± 0.60	26.1 ± 0.61	0.65 ± 0.14	0.88 ± 0.062	4.1 ± 0.26
Vincristine	12.5 ± 0.59	25.6 ± 0.44	0.96 ± 0.07	0.14 ± 0.048	4.9 ± 0.26
5-Fluorouracil	14.4 ± 0.42	21.8 ± 0.86	0.70 ± 0.21	0.67 ± 0.12	5.0 ± 0.36
Actinomycin-D	11.2 ± 0.21	29.5 ± 0.66	0.63 ± 0.20	0.32 ± 0.11	14.2 ± 0.63

* The results are the means and S.E. obtained from the livers of six rats except in the case of actinomycin and 5-fluorouracil where only five animals were available.
† nmole/mg protein/g liver/30 min.

affected to an even greater extent by prior administration of these cytotoxic drugs. Determination of absorption rates in cancer patients would be difficult in that it would involve a number of blood samples taken shortly after dosing with antipyrine and absorption is also complicated by many other factors [13]. Indeed variability in absorption rates in normal subjects has been known to complicate plasma half-life determinations for antipyrine [14]. However, any indication of the alteration in maximal absorption (or steady-state) values of antipyrine induced by cancer chemotherapeutic compounds could be of value in determining the condition of the gastrointestinal tract of the patient. Furthermore the rat might serve as a useful model for predicting the likely effect on absorption of some of the new cytotoxic drugs, and drug combinations being evaluated for cancer therapy.

The elimination rate of antipyrine from the plasma of man could be determined more conveniently than the absorption rate. The results obtained in this experiment with rats, where the plasma half-life of antipyrine was prolonged, provide an indication of what might occur in patients being treated with similar anti-cancer drugs. Drugs known to stimulate liver metabolism increase the rate of elimination of antipyrine from plasma [15]. The results obtained with these anti-cancer drugs, however, are more comparable with those associated with various forms of liver disease [1]. In liver disease the prolongation of antipyrine clearance time is generally ascribed to either a reduction in the ability of each hepatic cell to eliminate the drug, to a reduced functional cell mass or to a reduced perfusion of each cell with the drug. The results suggest that neither liver perfusion or cell mass was significantly affected by the anti-cancer drugs. The phenolphthalein excretion results obtained with the bile duct cannulated animals complement those obtained with antipyrine and suggest that the decrease in hepatic function was a more important factor in the observed impaired plasma clearance of antipyrine than was the delayed absorption of the drug from the gut.

However, in this experiment there was no correlation between the retardation *in vivo* of antipyrine clearance and an apparent increase in aryl hydrocarbon hydroxylase activity caused by administration of the anti-cancer drugs. Using rabbits a decrease in antipyrine hydroxylase activity *in vitro* has been correlated with a decrease in plasma elimination rate of antipyrine *in vivo* [16]. In this experiment the observed decrease in cytochrome P450 level caused by the anti-cancer drugs could have limited the rate of antipyrine clearance *in vivo*, but there is little evidence reported of such a correlation except in severe hepatic dysfunction [17]. There is more than one species of cytochrome P450 [18] and use of comparisons of P450 levels in rat to predict metabolism *in vivo* in man is further complicated in that man has less P450, but not necessarily P450-reductase activity.

The results *in vivo* suggest, however, that the determination of the plasma elimination of antipyrine before and after treatment with anti-cancer drugs provide a direct indication of the metabolic status of the liver as a result of the therapy, since each patient could serve as his or her own control.

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