ORIGINAL ARTICLE

INHIBITION OF HEPATIC DRUG METABOLISM BY ANTICANCER AGENTS

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SUMMARY

Pretreatment of the rats with methotrexate, cyclophosphamide and 5-fluorouracil could cause inhibition of hepatic drug metabolism of zoxazolamine, aminopyrine and aniline, as evidenced by a prolongation of zoxazolamine paralysis time and a decrease in the elimination of the latter two amines from the plasma. However, the rate of absorption of both aminopyrine and aniline were unaffected. *In vitro* investigations were consistent with the view that the most likely cause of this inhibited hepatic drug metabolism produced by these anticancer agents was the reduction in one or more components of the drug-metabolizing enzyme system, including the cytochrome P-450 content.

Chemotherapy plays a very important role in the treatment of neoplastic diseases. Single drug or several drugs in combination have been successfully employed either alone or in combination with surgery or radiotherapy. The therapy is mostly palliative but not curative (1). However, the majority of drugs used are highly toxic and also affect rapidly growing cells other than those of the neoplastic process. As a consequence, their therapeutic index is low.

Many studies have reported that anticancer drugs are cytotoxic to liver cells. Hepatitis, cirrhosis, fibrosis and fatty change in liver cells have been found in liver biopsy samples from psoriatic patients treated chronically with methotrexate (2). In the study of

Custer et al. (3) it was found that rats given more than 125 μg/kg methotrexate intraperitoneally five times per week for 24 months developed serious liver damage-namely, varying degrees of fatty metamorphosis, necrosis, atrophy of hepatic cord and fibrosis. Liver toxicity caused by cyclophosphamide is also well documented but 5-fluorouracil-induced hepatotoxicity is rare.

Relatively little is known of the effects of anticancer drugs on the action, metabolism and toxicity of other drugs used concomitantly in cancer patients. Decreased activities of hepatic microsomal drug-metabolizing enzymes have been reported in rats treated with 5-fluorouracil, cyclophosphamide, methotrexate, mechlorethamine, 6-mercaptopurine, or daunorubicin (4-7). The basis for these decreased enzymatic activities has not been fully elucidated.

The objective of this study is to determine the effects of subacute treatment of methotrexate, cyclophosphamide and 5-fluorouracil on hepatic drug-metabolizing enzymes.

MATERIALS AND METHODS

Experimental Animals and Chemicals

Adult male Fisher rats of about 60 days of age and weighing 170-200 g were used in the study. The animals were supplied by the Animal Center of the Faculty of Science, Mahidol University, Bangkok. Four rats were kept in one cage suspended over Absorb-Dri. All animals were allowed access of food (Purina Laboratory Chow, Zuellig Pte, Ltd., Singapore) and tap water ad libitum until 16 hours before sacrifice, during which time they were allowed access to water only.

Chemical agents used in this experiment were obtained commercially in the highest grade available without further purification.

Treatment of Experimantal Animals

The experimental animals were fasted overnight prior to treatment with the anticancer agents, which were freshly prepared just before use as a suspension in normal saline. All drugs were given to the animals in the morning around 8.00 to 9.00 A.M. After having been given the drugs, the rats were again fasted for about 16 hours before sacrifice.

1. Subacute experiments

The animals were intubated once daily for 5 consecutive days with methotrexate (1, 2, 3 and 5 mg/kg) and cyclophosphamide (20, 40 and 60 mg/kg) and the others were injected intraperitoneally with 5-fluorouracil (10, 20 and 30 mg/kg). Control animals received an identical treatment with normal saline solution. The animals were weighed and sacrificed 48 hours after last dose the drug.

2. Recovery experiments

The animals were treated orally with 2 mg/kg methotrexate and 40 mg/kg cyclophosphamide and intraperineally with 30 mg/kg 5-fluorouracil, once daily for 5 days. They were killed at 2, 4, 6, 10, 15 and 20 days after the last dose of the 5-day pretreatment schedule.

3. Collection of blood samples

Blood samples were collected from rats by heart puncture under light ether anesthesia. Heparin sodium solution (200 units per ml blood) was preintroduced into the syringe to prevent blood coagulation. The blood was then centrifuged at 3,000 rpm for 20 min in the International Centrifuge Size 2 Model K and the plasma was removed with a pasteur pipette. The plasma was kept under deep freeze at -20° C for further quantitative determination of drugs.

Analytical Assays

In the assay of aminopyrine N-demethylase or aniline hydroxy-

lase, the hepatic postmitochondrial fraction was used as enzyme source. In both cases, however, enzymatic activity was expressed per mg microsomal protein. Both the enzyme source and the microsomes were prepared by the method described by Unchern and Thithapandha (8).

The activities of aminopyrine N-demethylase and aniline hydroxylase were determined by the methods described by Mazel (9), except that 20 μmole of aminopyrine or 10 μmole of aniline and 1 μmole of NADP were used.

Microsomal protein was determined by the method of Lowry et al (10) with bovine serum albumin as the standard. Cytochrome P-450 in the microsomal pellet was estimated by the method of Omura and Sato(11).

The amount of aminopyrine in the plasma was determined by the method described by Brodie et al (12). Palsma aniline concentration was measured by the method of Kupfer and Bruggeman (13).

Zoxazolamine Paralysis Time

Zoxazolamine paralysis time was determined by measuring the time interval between the onset of paralysis and the regain of the movement after an intraperitoneal administration of 60 mg/kg zoxazolamine. Zoxazolamine solution was freshly prepared at the concentration of 20 mg/ml, according to the method described by Conney et al (14).

Pharmacokinetic Analysis and Statistical Analyses

All pharmacokinetic parameters obtained were computed according to the one-compartment open model (15).

The results are reported as the mean value ± S.E. The statistical significance was analyzed by multiple comparison using the method of Newman and Keul's as described by Grimm (16) with the level of significance $p < 0.05$. The method of least square was employed to draw all regression lines for pharmacokinetic data.

RESULTS

As shown in Table 1, pretreatment of rats with methotrexate, cyclophosphamide and 5-fluorouracil at the doses indicated produced a certain degree of toxicities to the animals, as evidenced by the formation of blood stool and a significant reduction in body weight. Many of them died a few days later.

After the animals had been pretreated with these drugs, even at the reduced doses, zoxazolamine paralysis time were markedly prolonged when compared with the control (Table 2). These results thus suggested that the anticancer drugs might inhibit the hepatic microsomal metabolism of the muscle relaxant. When the effects of each drug were investigated further, it was found that methotrexate depressed the enzymatic activities of both aminopyrine N-demethylase and aniline hydroxylase in a somewhat dose-dependent manner (Table 3). It also caused a dose-dependent reduction in hepatic cytochrome P-450 content,

Table 1. The effect of anticancer agents on rat body weight

	Body Weight (g)			
	Control	Metho-trexate	Cyclophos-phamide	5-Fluoro-uracil
Before treatment	166 ± 3.2	179 ± 7.2	184 ± 3.9	178 ± 4.7
After treatment	175 ± 2.8*	164 ± 5.5*	155 ± 5.3*	148 ± 8.5*

Six rats in each group were treated with the drug once daily for 5 days as follows : methotrexate (5 mg/kg, PO) ; cyclophosphamide (60 mg/kg, PO) ; and 5-fluororacil (30 mg/kg, IP).
Control animals received an equal volume of saline. The asterisk indicates a significant difference between before and after treatment ($P < 0.005$).

Table 2. The effect of methotrexate, cyclophosphamide and 5-fluorouracil pretreatment on zoxazolamine paralysis time

Pretreatment	Paralysis Time (min)
Control	45 ± 3
Methotrexate	83 ± 6*
Cyclophosphamide	119 ± 5*
5-Fluorouracil	141 ± 10*

Male rats weighing 170-200 g were pretreated once daily for 5 days with methotrexate (2 mg/kg, PO), cyclophosphamide (40 mg/kg, PO) and 5-fluorouracil (30 mg/kg, IP). Control animals received an equal volume of saline. Zoxazolamine (60 mg/kg) was injected (i.p.) about 50 hours after the last dose of the pretreatment; the paralysis time was then recorded. Results are expressed as mean ± S.E. from 7 rats. * $P < 0.01$ (from control; Newman-Keule' test)

while the liver weight and the microsomal protein level were unaffected (Table 4). The effects of cyclophosphamide on cytochrome P-450 content and the activities of these two drug-metabolizing enzymes qualitatively similar to those of methotrexate (Table 4), except that this alkylating agent also decreased microsomal protein content even at a low dose. 5-Fluorouracil at low doses had no effect on all of the above parameters though physically the animals appeared to be sick (Table 5). However, at a higher dose such as 30 mg/kg the drug could decrease all of these parameters except the liver weight.

The effects of methotrexate, cyclophosphamide and 5-fluorouracil seemed to be long lasting inasmuch as it took more than 20 days after the drug's last dose for aniline hydroxylase activity to return to normal (Fig. 1). Many of the treated rats died during this course of enzymatic recovery. When they died their drug-metabolizing activity

Table 3. Effects of methotrexate on various parameters of drug-metabolizing enzyme systems

Methotrexate (PO, Once daily for 5 days) (mg/kg)	Liver Weight (% of Body Weight)	Microsomal Protein (mg/g liver)	Aminopyrine N-Demethylase Activity[a]	Aniline Hydroxylase Activity[b]	Cytochrome P-450 (nmole/mg protein)
0	3.00 ± 0.05	31.80 ± 0.31	137.50 ± 1.79	14.15 ± 0.30	0.56 ± 0.01
1	3.02 ± 0.09	31.06 ± 0.50	121.00 ±10.70	11.98 ± 1.61	0.57 ± 0.02
2	3.97 ± 0.23	28.46 ± 1.06	68.54 ±12.57*	4.52 ± 0.75*	0.38 ± 0.03*
3	3.94 ± 0.17	29.14 ± 0.46	58.55 ± 9.75*	4.03 ± 0.87*	0.32 ± 0.03*
5	3.58 ± 0.09	31.09 ± 1.08	21.43 ± 2.15*	2.25 ± 0.20*	0.24 ± 0.03*

Values are expressed as mean ± SE from 6 determinations
a = nmole formaldehyde formed / mg protein / 30 min.
b = nmole p-aminophenol formed / mg protein / 20 min.
Asterisks indicate significant difference from control ($P < 0.05$; Newman-Keuls' test)

Table 4. Effects of cyclophosphamide on various parameters of drug-metabolizing enzyme systems

Cyclophosphamide (PO, Once daily for 5 days) (mg/kg)	Liver Weight (% of Body Weight)	Microsomal Protein (mg/g liver)	Aminopyrine N-Demethylase Activity[a]	Aniline Hydroxylase Activity[b]	Cytochrome P-450 (nmole/mg protein)
0	2.90 ± 0.08	38.98 ± 1.22	144.07 ± 6.61	13.77 ± 0.69	0.58 ± 0.02
20	2.87 ± 0.03	33.94 ± 0.85*	102.48 ± 4.27*	9.41 ± 0.68*	0.42 ± 0.06*
40	3.64 ± 0.18	31.57 ± 1.81*	83.73 ± 5.79*	5.16 ± 0.38*	0.28 ± 0.02*
60	4.02 ± 0.14	28.97 ± 1.23*	60.84 ± 7.46*	3.46 ± 0.30*	0.21 ± 0.01*

Values are expressed as mean ± SE from 6 determinations
a = nmole formadehyde formed/mg protein/30 min.
b = nmole p-aminophenol formed/mg protein/20 min.
Asterisks indicate significant difference from control ($P < 0.05$; Newman-Keuls' test)

Table 5. Effects of 5-fluorouracil on various parameters of drug-metabolizing enzyme systems

5-FU (IP, Once daily for 5 days) (mg/kg)	Liver Weight (% of Body Weight)	Microsomal Protein (mg/g liver)	Aminopyrine N-Demethylase Activity[a]	Aniline Hydroxylase Activity[b]	Cytochrome P-450 (nmole/mg protein)
0	3.08 ± 0.06	27.23 ± 1.02	127.22 ± 9.53	13.36 ± 1.03	0.52 ± 0.03
10	3.15 ± 0.06	27.93 ± 1.93	118.36 ± 8.04	10.76 ± 0.90	0.48 ± 0.03
20	2.97 ± 0.04	27.70 ± 1.59	120.53 ± 4.85	13.45 ± 0.54	0.55 ± 0.02
30	3.48 ± 0.10	22.50 ± 0.88*	59.77 ± 5.45*	7.36 ± 1.11*	0.32 ± 0.04*

Values are expressed as mean ± SE from 6 determinations.
a = nmole formaldehyde formed/mg protein/30 min.
b = nmole p-aminophenol formed/mg protein/20 min.
Asterisks indicate significant difference from control ($P < 0.05$; Newman-Keuls' test)

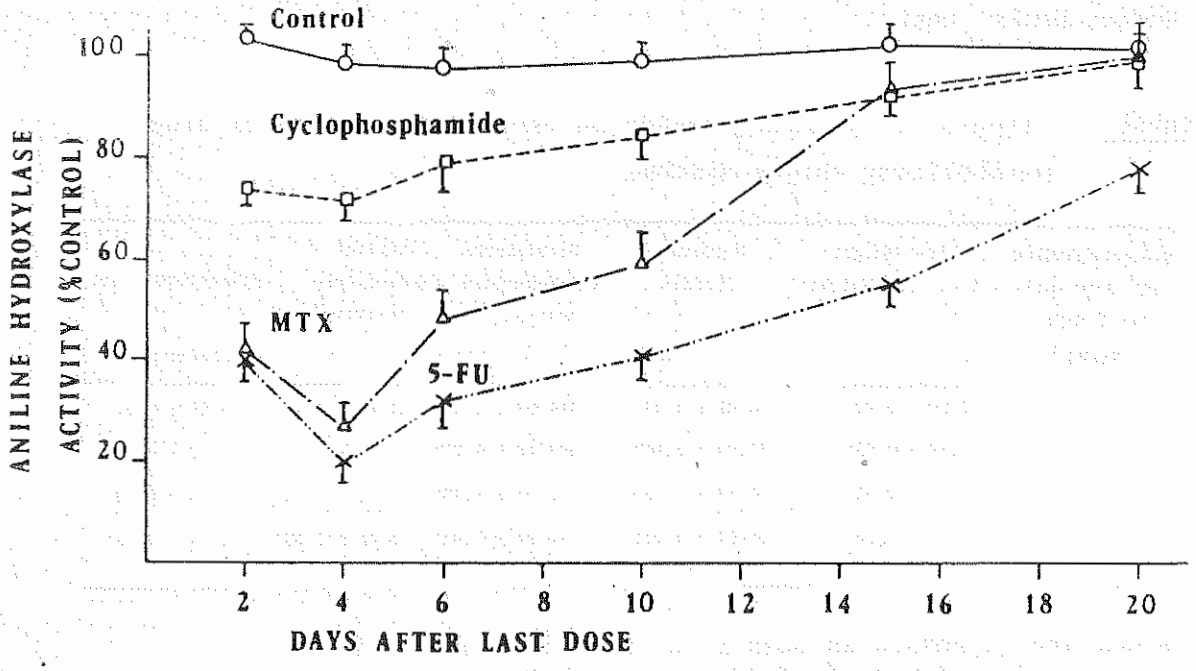

Figure 1. Time-course of the effects of anticancer agents on aniline hydroxylase activity. Methotrexate (2 mg/kg, PO), cyclophosphamide (40 mg/kg, PO), and 5-fluorouracil (30 mg/kg, IP) were given once daily for 5 days as described previously. Each point represents mean ± S.E. from 6 separate determinations.

(aniline hydroxylase) was still low, approximately 20-40 % of control. Similar results were also obtained with aminopyrine N-demethylase (Fig. 2).

The plasma concentration-time curves of aminopyrine and aniline in control and drug-treated animals are shown in Figs. 3 and 4; their pharmacokinetic parameters are tabulated in Tables 6 and 7 . All of the three anticancer drugs caused an increase in both the plasma half-life and volume of distribution of the two tested amines. The drugs did not seem to cause any change in the initial rates of absorption of aminopyrine though they did produce somewhat lower peak levels of aniline in the treated groups (Figs. 3 and 4).

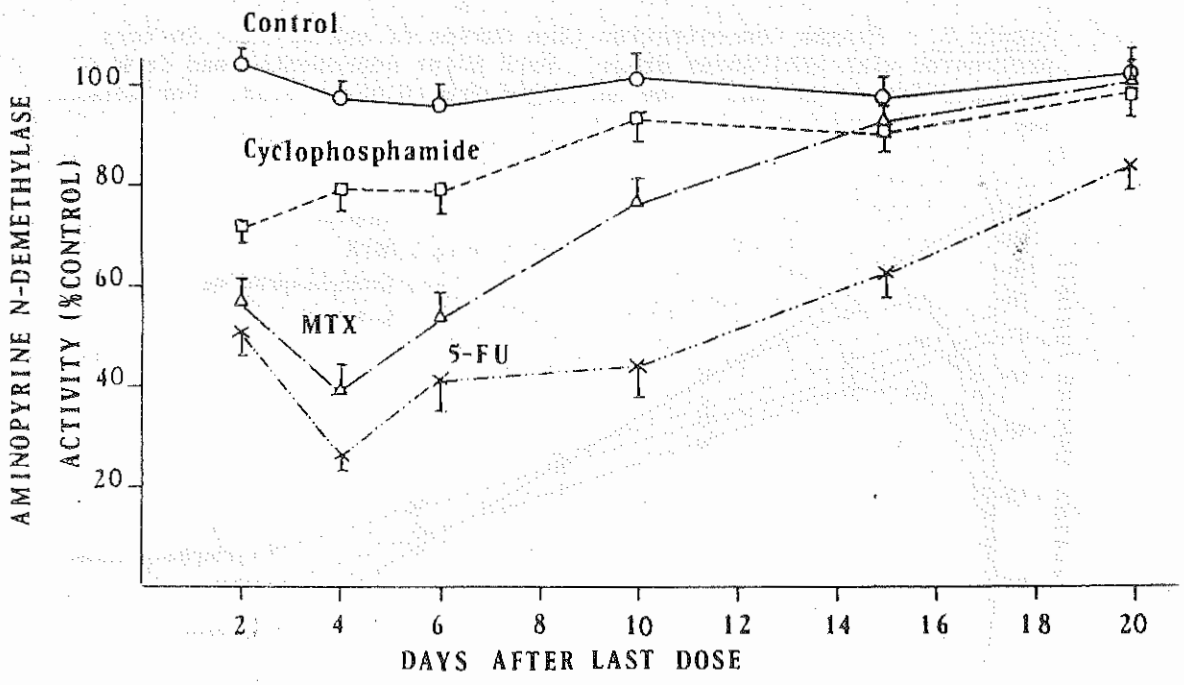

Figure 2. Time-course of the anticancer agents on aminopyrine N-demethylase activity. Methotrexate (2 mg/kg, PO), cyclophosphamide (40 mg/kg, PO) and 5-fluorouracil (30 mg/kg, IP) were given once daily for 5 days as described previously. Each point represents mean ± S.E. from 6 separate determinations.

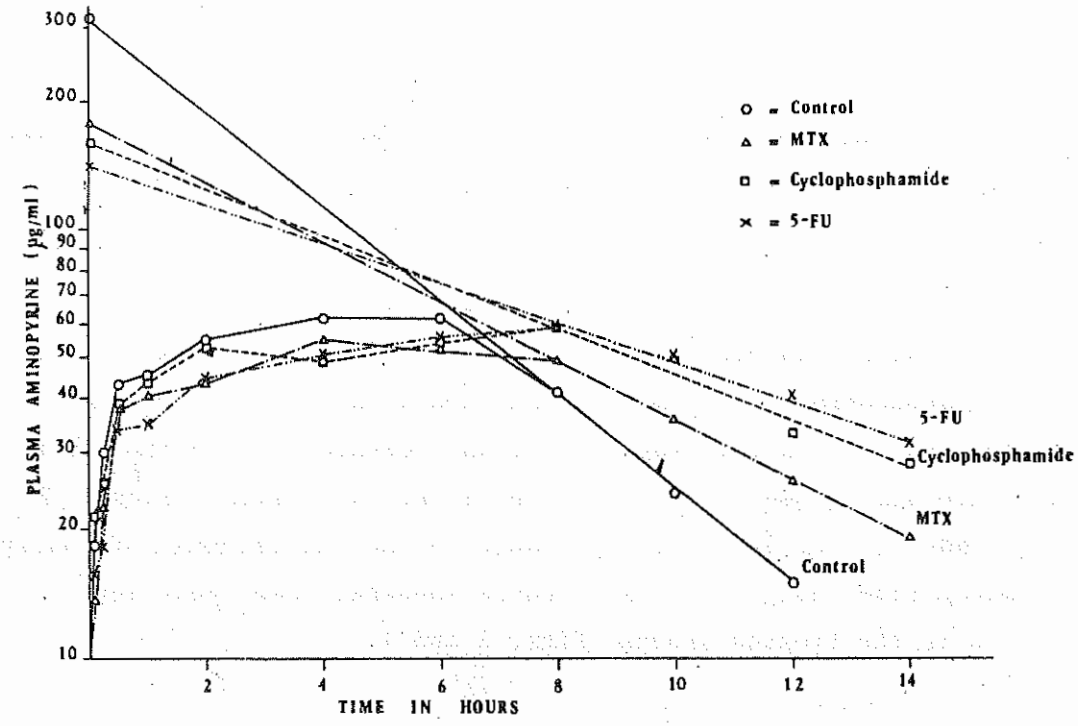

Figure 3. Plasma concentration-time curves of aminopyrine in rats pretreated with anticancer drugs. Each point represents mean from 4 separate determinations. The SE. ranged from 0.63 to 8.33. For detail, see Table 6.

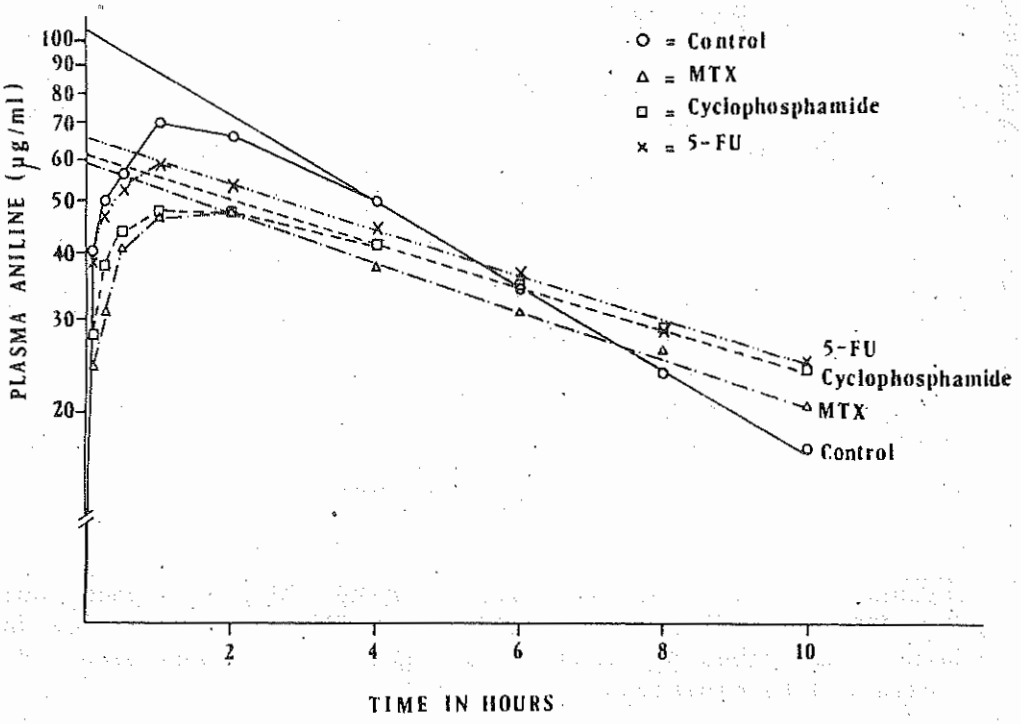

Figure 4. Plasma concentration-time curves of aniline in rats pretreated with anticancer drugs, Each point represents mean from 4 separate determinations. The SE. ranged from 0.56 to 4.78. For detail, see Table 7.

Table 6. The pharmacokinetics of aminopyrine in animals pretreated with anticancer agents

Pretreatment	Half-life (hrs)	Plasma Clearance (L/kg/hr)	Vd (L/kg)
Control	2.84 ± 0.14	0.16 ± 0.008	0.65 ± 0.01
Methotrexate	4.42 ± 0.34*	0.18 ± 0.015	1.19 ± 0.15*
Cyclophosphamide	5.56 ± 0.08†	0.15 ± 0.017	1.27 ± 0.31†
5-Fluorouracil	7.60 ± 1.20†	0.14 ± 0.008	1.49 ± 0.28†

Male rats weighing 170-200 g were pretreated once daily for 5 days with methotrexate (2 mg/kg, PO), cyclophosphamide (40 mg/kg, PO), and 5-fluorouracil (30 mg/kg, IP). Control animals received an equal volume of normal saline. Aminopyrine (200 mg/kg) was given orally 48 hours after the last dose of pretreatment. The animals were then sacrified at various time intervals by heart puncture under light ether anesthesia. The plasma level of aminopyrine and the pharmacokinetic constants were determined as described for once-compartment open model, with the assumption that aminopyrine was completely absorbed in all groups. The results are expressed as mean ± SE from 4 separate determinations. * $P < 0.05$ (from control) ; † $P < 0.01$ (from control)

DISCUSSION

It is not uncommon to give drugs to cancer patients who have already been treated with cancer chemotherapeutic agents with little regard to the possible hepatotoxic actions of these compounds. The results obtained in the present study that pretreatment of rats with methotrexate, cyclophosphamide and 5-fluorouracil could prolong zoxazolamine paralysis time and increase the plasma half-life of both aminopyrine and aniline (Table 2, 6 and 7; Figs 3 and 4) provide a good evidence that anticancer agents at appropriate dose can have a profound effect on hepatic drug metabolism. One might regard that the experi-

Table 7. The pharmacokinetics of aniline in animals pretreated with anticancer agents

Pretreatment	Half-life (hrs)	Plasma Clearance (L/kg/hr)	Vd (L/kg)
Control	3.83 ± 0.29	0.17 ± 0.003	0.95 ± 0.06
Methotrexate	7.13 ± 1.22*	0.17 ± 0.011	1.68 ± 0.19*
Cyclophosphamide	7.54 ± 0.80*	0.15 ± 0.007	1.63 ± 0.11*
5-Fluorouracil	6.10 ± 0.22*	0.15 ± 0.004	1.46 ± 0.60*

Male rats weighing 170-200 g were treated once daily for 5 days with methotrexate (2 mg/kg, PO), cyclophosphamide (40 mg/kg, PO) and 5-fluorouracil (30 mg/kg. IP). Control animals received an equal volume of normal saline. Aniline hydrochloride (100 mg/kg) was given orally 48 hours after the last dose of the drug treatment. The animals were then sacrified at various time intervals by heart puncture under light ether anestesia. The plasma levels of aniline were measured as described in Materials & Methods. The pharmacokinetic constants were obtained as described for the one-compartment open model, with the assumption that aniline was completely absorbed in all groups. The results are expressed as mean ± SE from 4 separate determinations.
* $P < 0.05$ (from control)

ments with anticancer agents are comparable with those associated with various forms of liver disease. In liver disease the prolongation of drug action or increase in the drug's plasma half-life is generally ascribed to either a reduction in the ability of hepatic cells to eliminate the drug, to a reduced functional cell mass or to a reduced perfusion of each cell with the drug. The *in vitro* results on the effects of methotrexate, cyclophosphamide and 5-fluorouracil on various hepatic parameters (Tables 3-5) were complementary to those obtained with aminopyrine or aniline pharmacokinetics (Tables 6 and 7) and sug-

gested that decrease in hepatic drug metabolism was a key factor in the observed impaired plasma clearance of the above two amines. Delayed absorption of either aminopyrine or aniline from the gut was not likely to be the cause since the plasma concentration-time profiles of either amine during the absorption phase in control and drug-pretreated groups were very similar (Figs. 3 and 4).

However, these three anticancer agents at the doses employed in the present study did not cause any change in SGPT and SGOT levels (data not shown). Thus, there was no correlation between the transaminase activity and the abillity of the liver to metabolize drugs.

The results obtained from this study are similar to those of Capel et al. (17), who found that pretreatment of rats with 5 anticancer drugs retarded and decreased absorption from the gastrointestinal tract and the elimination of antipyrine from the plasma; the latter effect was believed to be due to a reduction in hepatic cytochrome P-450 content. In the present study, however, the effect of anticancer drugs on the absorption profile of either aminopyrine or aniline was not as clear cut as it was found with antipyrine by the above authors. The discrepancy has yet to be settled.

The effect of anticancer drugs on hepatic drug metabolism may have clinical relevance. Drug dosage should be modified on cancer patients receiving chemotherapy. Capel et al. (17) have suggested that antipyrine be used in patients before and after treatment with anticancer drugs to obtain a direct indication of the metabolic status of the liver as a result of the therapy. This method seems reliable since each patient can serve his or her own control. Moreover, in view of the fact that anticancer drugs may be given to patients in combination their effects on liver function could be more serious. Without dosage adjustment several high-risk drugs may produce a fatal outcome in these patients.

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