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THE EFFECT OF ANTI-CANCER DRUGS ON PHARMACOKINETICS OF ANTIPYRINE IN VITAMIN A DEFICIENCY

K. L. KHANDUJA*, S. C. DOGRA, SARITA KAUSHAL and R. R. SHARMA

Department of Biophysics, Postgraduate Institute of Medical Education and Research, Chandigarh-160012, India

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Abstract—The pharmacokinetics of orally and intravenously administered antipyrine-^{14}C in individual rats were investigated to examine the effects of vitamin A deficiency and/or cytotoxic anti-cancer drugs, like cyclophosphamide, methotrexate, 5-fluorouracil and actinomycin-D. Both vitamin A deficiency and pretreatment of rats with anti-cancer drugs led to impairment in the absorption from the gastro-intestinal tract and the plasma elimination of antipyrine. Pretreatment of vitamin A deficient rats with anti-cancer drugs further retarded the absorption and plasma clearance of antipyrine. Apparent volume of distribution of antipyrine was reduced ($P < 0.001$) by inducing vitamin A deficiency in rats, whereas pretreatment of rats with anti-cancer drugs did not show any effect.

Cytotoxic drugs, and vitamin A deficiency increased the area under the plasma concentration vs. time curve of orally administered antipyrine (AUC_{oral}). *In vitro* findings suggest that the most probable cause of delayed clearance/prolonged half-life and increased AUC_{oral} values was the reduction in hepatic cytochrome P-450 dependent mixed function oxidase system.

Vitamin A deficiency is an important public health problem. Certain population groups show less than optimal vitamin A nutritional status. It has also been observed that persons who eventually develop cancer have lower serum vitamin A levels as compared to the normal population [1]. In addition to the vital role of vitamin A in maintaining the normal epithelial structure, its altered levels in the body affect drug metabolizing capacity of the liver *in vitro* [2–4] and *in vivo* [2]. However, *in vivo* studies are not sufficient in number to support the *in vitro* observations. Also, vitamin A deficiency results in degeneration of mucus-secreting cells of gastro-intestinal (GI) tract, whereas in other tissues mucus-secreting cells get replaced by squamous keratanizing epithelium [5].

Antipyrine (AP) is a widely used drug in man as well as in experimental animal models to evaluate liver function and possibly the pharmacokinetics of other administered drugs. Studies of Capel *et al.* [6] have shown that pretreatment of rats with anti-cancer (AC) cytotoxic drugs decreased and delayed the absorption of antipyrine from GI tract. Plasma elimination of antipyrine was also found to be delayed. Later on, Hoyem-Johansen *et al.* [7] showed a different finding in this regard. In the present study, we have investigated the effect of vitamin A deficiency, and the pretreatment of vitamin A deficient rats with anti-cancer drugs on GI absorption and plasma clearance of antipyrine. In addition, the above-mentioned controversy of pharmacokinetics of antipyrine in anti-cancer drugs pretreated rats has also been re-evaluated.

* To whom all correspondence and reprint requests should be addressed.

MATERIALS AND METHODS

(*A*) *Drugs.* Antipyrine, retinyl-acetate, cyclophosphamide, 5-fluorouracil, methotrexate and actinomycin-D were purchased from Sigma Chemical Co., U.S.A., Antipyrine-*N*-methyl-^{14}C (sp. act. 50 mCi/mMol) was purchased from Amersham, England. All other chemicals of analytical grade were purchased locally. 3-Hydroxybenzo(*a*)pyrene was generously supplied by Prof. J. N. Keith, IIT, Chicago, U.S.A.

(*B*) *Preparation of animals.* Male weanling Wistar rats (50–60 g) of the institute's colony were used, and were divided into two groups. The first group was maintained on vitamin A deficient casein based diet [8] for 4 weeks. The second group was pair-fed with additional oral feeding of 700 i.u. retinyl-acetate/rat twice a week. Animals had free access to water and food during 4 weeks, and thereafter each group was further divided into two sub-groups, each consisting of 5 animals. The diet supplied to pair-fed group was adjusted in proportion to that consumed by the deficient group. Rats of two sub-groups, one out of each main group, were given either an oral dose of cyclophosphamide (4 mg/kg) or methotrexate (1 mg/animal) in saline (2 ml/kg) once daily for 5 successive days, or i.p. administration of actinomycin-D (0.1 mg/kg) or 5-fluorouracil (12.5 mg/kg) in saline (2 ml/kg) for two successive days. The other two sub-groups received the similar treatment with saline only. One day after cessation of the treatment, animals were kept on overnight fasting in wide mesh cages so as to prevent them from caprophagy. On the following day, animals of each sub-group received an oral dose or i.v. administration of antipyrine-^{14}C (18 mg/kg, 18 μCi/kg) in saline (2 ml/kg). Blood samples (approx. 0.1 ml) were collected from rats, under light ether anaes-

thesia, by occular vein puncture with dilute heparin flushed glass capillary at 5, 10, 15, 30, 60 min and 2, 3, 4 and 6 hr after dosing. Biological half-life ($T_{\frac{1}{2}}$), apparent volume of distribution (V_d) and clearance rate (CL) of the drug were estimated after i.v. administration of antipyrine [9]. AUC_{oral} was calculated as described in the literature [7].

(*C*) *Radioactivity counting*. The blood sample, 0.1 ml, was vortex mixed with 2 ml of dioxan and centrifuged at 2500 *g* for 15 min. One ml of clear organic phase was counted in 10 ml of scintillation fluid consisting of 5 g PPO, 100 mg POPOP and 100 g naphthalene in 1 l. of toluene:dioxan mixture (1:1 v/v). Quenching was determined by external standardization. Under identical extraction procedures more than 99% of radioactivity due to antipyrine-^{14}C was found to be extractable.

(*D*) *Enzyme assays*. Liver microsomes were separated by ultracentrifugation as described earlier [4], except that instead of KCl/Tris–HCl buffer, 50 mM phosphate buffer was used. Protein was estimated by the method of Lowry *et al.* [10], using bovine serum albumin as standard. Cytochrome P-450 was estimated by the method of Omura and Sato [11]. Aryl hydrocarbon hydroxylase (AHH) was determined by the method of Nebert and Gelboin [12]. Vitamin A contents in the liver and intestine were determined by the method of Dugan *et al.* [13].

Statistical analysis was done by Student's *t*-test.

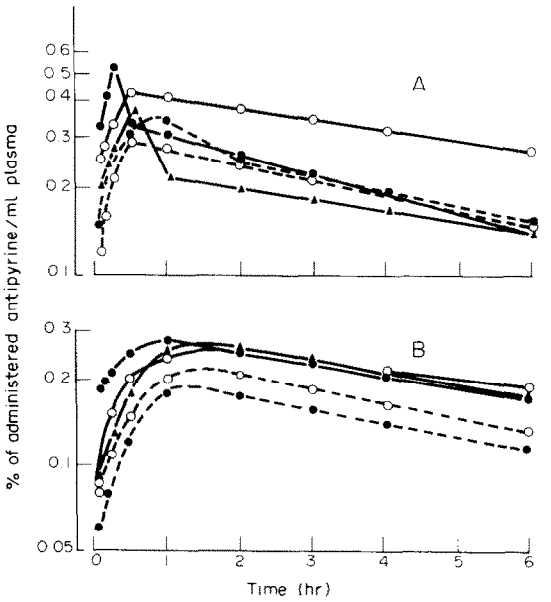

Fig. 1. Effect of pretreatment with saline (-●-●-), cyclophosphamide (-▲-▲-), 5-fluorouracil (--●---●--), methotrexate (--○--○--) and actinomycin-D (-○—○-) on plasma time–concentration curve of orally administered antipyrine-^{14}C (18 mg/kg) in pair-fed (A) and vitamin A deficient (B) rats.

RESULTS

In vivo *studies*

Vitamin A deficient rats had body growth and liver weight similar to pair-fed animals. Hepatic and intestinal vitamin A contents in pair-fed rats were 86.46 ± 9.24 μg/g and 3.5 ± 0.52 μg/g respectively; whereas in vitamin A deficient animals values were 1.4 ± 0.5 μg/g and not detectable respectively.

A concentration–time curve of AP, following its oral administration for each subgroup is shown in Fig. 1. Each time point in Fig. 1 represents the mean of 5 determinations. The maximal plasma level of AP in pair-fed untreated rats was achieved within 15 min, whereas in vitamin A deficient untreated rats it occurred by 1 hr. Pretreatment of rats with AC drugs delayed the achievement of maximal plasma level of antipyrine by 15 min to 45 min in pair-fed rats, and by 0–1 hr in vitamin A deficient rats. Vitamin A deficiency not only delayed the occurence of maximal plasma level but also reduced the level of maximal concentration of AP. Pretreatment of vitamin A deficient rats with AC drugs further delayed the absorption, except in case of methotrexate, and significantly ($P < 0.05$) reduced the maximal plasma concentration of AP in methotrexate and 5-fluorouracil treated rats.

Vitamin A deficiency and/or pretreatment with AC drugs delayed the absorption of AP. Therefore, in another study rats were i.v. injected AP at the similar dose to find out the metabolic fate of the drug *in vivo*, i.e. to find out the exact values of its $T_{\frac{1}{2}}$, CL, and V_d. Vitamin A deficiency caused a significant decrease in CL and V_d ($P < 0.001$) and a significant increase in $T_{\frac{1}{2}}$ and AUC_{oral} ($P < 0.001$) (Table 1). Pretreatment of pair-fed rats with AC drugs enhanced $T_{\frac{1}{2}}$ and AUC_{oral} significantly ($P < 0.05$) without affecting V_d. In vitamin A deficient rats $T_{\frac{1}{2}}$ was further increased by the pretreatment of animals with the AC drugs. Cyclophosphamide and actinomycin-D did not change the basal values of AUC_{oral} in vitamin A deficient rats, whereas 5-fluorouracil and methotrexate caused a significant decrease.

In vitro *studies*

Vitamin A deficiency did not affect the microsomal protein content of the liver, whereas the levels of cytochrome P-450 and AHH were decreased (Table 2). Cyclophosphamide pretreatment in pair-fed rats enhanced the microsomal protein contents ($P < 0.05$). However, all other drugs did not affect the liver microsomal protein contents both in pair-fed and vitamin A deficient rats. All the used AC drugs significantly lowered the levels of cytochrome P-450 and AHH in pair-fed animals. Similar was the effect in vitamin A deficient rats, except in the case of methotrexate which did not change the level of AHH.

DISCUSSION

Pretreatment of rats with AC drugs reduced the rate of enteral AP absorption from GI tract. These findings are in agreement with the observations of Capel *et al.* [6]. However, these observations differ from the study of Hoyem-Johansen *et al.* [7] who noticed the maximal absorption within 3–6 min in control rats and the delayed absorption only in the case of pretreatment with methotrexate. In our study, in spite of the same dose regimens, the max-

Table 1. Effect of pretreatment with anti-cancer drugs on effective half-life ($T_{\frac{1}{2}}$), clearance rate (CL), apparent volume of distribution (V_d) and AUC_{oral} of antipyrine in pair-fed and vitamin A deficient rats (values are given as $\bar{x} \pm$ S.D., $n = 5$)

Treatment		$T_{\frac{1}{2}}$ (min)	CL (ml min^{-1}kg^{-1})	V_d (ml kg^{-1})	AUC_{oral} (mg min kg^{-1})
Saline	I	225 ± 30	2.09 ± 0.19	680 ± 42	25.83 ± 1.3
	II	325 ±27^{a3}	1.18 ±0.18^{a3}	550 ±20^{a3}	36.02 ±1.73^{a3}
Cyclophosphamide	I	330 ±45^{a2}	1.40 ±0.26^{a2}	665 ± 48	30.05 ±1.54^{a2}
	II	415 ±20^{b3,c2}	0.91 ±0.06^{b1,c2}	545 ±35^{c2}	33.62 ± 1.82
5-fluorouracil	I	345 ±30^{a3}	1.34 ±0.12^{a3}	670 ± 25	32.51 ±1.61^{a3}
	II	440 ±36^{b3,c2}	0.85 ±0.09^{b2,c3}	535 ±28^{c3}	23.28 ±1.36^{b3,c3}
Methotrexate	I	275 ±30^{a1}	1.63 ±0.16^{a2}	650 ± 40	29.45 ±1.54^{a2}
	II	380 ±20^{b2,c2}	1.06 ±0.08^{c3}	580 ±30^{c1}	24.23 ±1.31^{b3,c2}
Actinomycin-D	I	360 ±45^{a3}	1.38 ±0.11^{a3}	715 ± 55	56.43 ±4.72^{a3}
	II	460 ±40^{b3,c2}	0.79 ±0.06^{b2,c3}	525 ±35^{c3}	39.21 ±3.6^{c3}

I: Pair-fed rats.
II: Vitamin A deficient rats.
a_1, a_2, a_3: Saline treated vitamin A deficient rats, and pair-fed rats pretreated with AC drugs compared with saline treated pair-fed rats.
b_1, b_2, b_3: Vitamin A deficient rats pretreated with AC drugs compared with vitamin A deficient saline-treated rats.
c_1, c_2, c_3: Vitamin A deficient rats pretreated with AC drugs compared with pair-fed rats pretreated with AC drugs.
Statistical significance: a_1, b_1, c_1, $P < 0.05$; a_2, b_2, c_2, $P < 0.01$; a_3, b_3, c_3, $P < 0.001$.

Table 2. Effect of pretreatment with anti-cancer drugs on livers of pair-fed and vitamin A deficient rats (values are given as $\bar{x} \pm$ S.D., $n = 5$)

Treatment		Microsomal protein (mg/g)	Cytochrome P-450 (nmol/mg protein)	AHH (pmol/min/mg)
Saline	I	18.2 ± 1.6	0.72 ± 0.06	203 ± 16
	II	17.6 ± 2.1	0.47 ± 0.04^{a3}	120 ± 18^{a3}
Cyclophosphamide	I	22.1 ± 1.1^{a2}	0.58 ± 0.05^{a2}	138 ± 19^{a3}
	II	20.3 ± 1.8	0.24 ± 0.08^{b3,c3}	95 ± 11^{b1,c2}
5-Fluorouracil	I	20.4 ± 1.4	0.52 ± 0.05^{a3}	141 ± 21^{a3}
	II	18.7 ± 0.9	0.30 ± 0.06^{b3,c2}	99 ± 9^{b1,c2}
Methotrexate	I	19.4 ± 0.9	0.59 ± 0.06^{a2}	175 ± 16^{a1}
	II	17.8 ± 0.8	0.38 ± 0.04^{b2,c3}	105 ± 8^{c3}
Actinomycin-D	I	20.6 ± 1.3	0.39 ± 0.06^{a3}	126 ± 16^{a3}
	II	18.4 ± 0.9	0.30 ± 0.05^{b3,c1}	92 ± 12^{b1,c2}

Notations are the same as in Table 1.

imal AP level in plasma of saline treated rats was achieved only by 15 min, which was further prolonged by pretreatment of rats with the AC drugs. At the end of *in vivo* studies animals were killed. We did not find any food or fecal material in the upper part of the GI tract, thus indicating that the longer starvation of animals could be the possible factor for different observations reported by Hoyem-Johansen.

It was interesting to note that, like pretreatment with AC drugs, vitamin A deficiency in animals also caused the reduction in rate of absorption and maximal plasma concentration of AP. It is likely that impaired absorption of AP through the GI route could be due to degeneration of mucus-secreting cells and impairment in mucosal blood flow and parietal cell function which regulate the clearance of drugs from the GI tract [14]. It is possible that these changes were further affected by the AC drugs. Clearance of orally administered AP from the plasma of pair-fed and vitamin A deficient rats pretreated with the cytotoxic drugs was impaired and it seems to be a net difference of plasma clearance and enteral absorption rates. Therefore, in another study, AP was injected i.v. to access the real clearance rate of AP in various conditions.

Clearance of AP from the plasma was reduced significantly ($P < 0.01$) by the pretreatment of pair-fed rats with AC drugs. A similar effect was noticed in vitamin A deficient rats, which was further enhanced by treating these animals with the cytotoxic drugs (Table 2). AP is metabolized by the microsomal cytochrome P-450 dependent mixed function oxidase system. The changes in clearance of this drug are finally depicted in terms of drug metabolizing enzymes. Vitamin A deficiency declined the hepatic level of AHH and cytochrome P-450 without affecting the microsomal protein contents (Table 2). Pretreatment of pair-fed animals with the cytotoxic drugs, except cyclophosphamide, did not change the microsomal protein contents, however, levels of both cytochrome P-450 and AHH were decreased. These findings are in contrast with the observations of Capel *et al.* [6], who had shown increased activity of AHH by treating their rats with cyclophosphamide, methotrexate and 5-fluorouracil. On the other hand, a decrease in antipyrine hydroxylase activity *in vitro* has been correlated with a decrease in plasma elimination rate of AP in rabbits [15]. Similar to pair-fed rats, treatment of vitamin A deficient rats with AC drugs, except methotrexate, caused a decrease in AHH levels ($P < 0.05$). Marinello *et al.* [16] have recently shown that the depression of hepatic drug metabolizing status by cyclophosphamide is due to the denaturation of microsomal cytochrome P-450 by cyclophosphamide metabolites. However, it remains to be elucidated if other cytotoxic drugs have a similar effect on hepatocyte.

AUC_{oral} values for AP were significantly increased by the treatment of animals with antineoplastic agents, agreeing with the earlier report [7], and also by inducing vitamin A deficiency. Whereas, treatment of deficient rats with the cytotoxic drugs, except cyclophosphamide and actinomycin-D, decreased the AUC_{oral} values. However, the common reason for all these changes seems to be the reduction in AP absorption from GI tract and/or its metabolism by the liver. Impairment in metabolism is important in drugs, such as cydophosphamide, which requires oxidative metabolism for the activity [16]. Besides this, intensity and duration of their cytotoxicity may be determined by the rate at which the cytotoxic moieties generated are metabolically detoxified. On the other hand, various authors have pointed out the importance of catabolic pathway of which the formation of dihydro-5-fluorouracil is the first step to explain therapeutic failures with 5-fluorouracil [17, 18].

All these drugs are repeatedly administered during chemotherapy as a single agent or in combination with other drugs. Therefore, their ability to interfere with hepatic drug metabolizing capacity may be of clinical importance. If there is a reduction in the drug metabolizing capacity then the level of active metabolites may be too low to cause sufficient tumor cell killing or the sustained presence of cytotoxic species may result in non-selective toxicity to host. In conclusion, this study indicates that the decreased drug absorption and metabolism due to pretreatment with cyclophosphamide, 5-fluorouracil, methotrexate and actinomycin-D in impaired body vitamin A status may be important in the chemotherapy of patients.

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