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INCREASE OF CHLORAMPHENICOL GLUCURONIDATION IN RATS TREATED WITH PHENOBARBITAL

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Abstract—After pretreatment of rats with 80 mg kg phenobarbital the elimination of chloramphenicol (CAP) from the blood increased considerably. The half-lifetime fell from 29 to 8 min. Concomitantly the concentration of conjugated CAP in the serum increased several fold. The level of active, unchanged CAP in the liver decreased whereas the amount of conjugated CAP increased, indicating that the accelerated elimination was caused by an increased conjugation rate. The availability of CAP administered orally was less than 50 per cent, even in phenobarbital pretreated rats.

IN A PREVIOUS study[1] it has been shown that after treatment of rats with phenobarbital the serum levels and the chemotherapeutic activity of chloramphenicol (CAP) are significantly reduced, and the amount of conjugated CAP in the urine is increased. This action of phenobarbital is due to an enhanced activity of the liver microsomal transferase which conjugates CAP by glucuronidation.[2] It might be possible that treatment of patients with drugs, such as phenobarbital, which are known to induce microsomal drug-metabolizing enzyme systems, enhance the conjugation of CAP, lower its serum level and diminish its activity.

We therefore tried to estimate in rats the magnitude of the phenobarbital effect so that the consequence of increased conjugation on the chemotherapeutic effect of CAP in human therapy could be evaluated. By comparing the elimination rates of the active CAP from the blood and determining the amount of the conjugated products in the serum and liver of rats we found a three- to four-fold increase of the CAP elimination after phenobarbital treatment.

MATERIAL AND METHODS

Male Wistar rats weighing 140–155 g were used in these experiments. The animals received 80 mg/kg of sodium phenobarbital intraperitoneally for 3 days in order to induce drug-metabolizing enzymes. After a 24 hr fasting period, following the last dose of phenobarbital, a single dose of chloramphenicol was administered by gastric intubation (100 mg/kg) or intravenous injection (30 mg/kg), as specified in each study. The oral preparation of chloramphenicol consisted of a suspension in 2% gum

arabic; for the intravenous administration a water solution of the glycine ester was used which is characterized by high solubility and rapid hydrolysis.[3,4]

At different times after the administration of CAP either five normal or five induced rats were exsanguinated and the serum collected for the quantitative determination of CAP and its metabolites. One gramme of liver from each rat was homogenized with 4 ml water for the determination of CAP and its metabolites.

The following methods of chemical assay for the antibiotic and its metabolites in serum and in liver were employed.

Active chloramphenicol. The method is based on an extraction in chloroform–ethyl acetate (2:1 by vol). Chloramphenicol was determined by the method of Levine and Fischbach.[5]

Chloramphenicol glucuronide. The conjugated fraction was hydrolyzed enzymatically by incubating serum or liver homogenate for 18 hr at pH 5·2 with 5000 Fishman units of β-glucuronidase (Ketodase, Warner–Chilcott) per 1 ml serum or 1 g liver. After the hydrolyses the samples were extracted with chloroform–ethyl acetate (2:1 by vol). The conjugated fraction equals the difference between total and active antibiotic.

Chloramphenicol reduced. The total arylamines were determined, after a preliminary acid hydrolysis at 100° for 1 hr by diazotization and coupling as described by Bratton and Marshall.[6]

RESULTS

The half-lifetime of unchanged CAP in the blood after i.v. injection decreased from 29 min in normal rats to 8 min in those pretreated with phenobarbital (Fig. 1). Extrapolation of both exponential disappearance curves back to zero time leads to the same concentration of 15 μg/ml indicating that phenobarbital treatment does not change the distribution of CAP in the body. The apparent distribution volume of CAP was 2, calculated from the ratio of dose (30 mg/kg) to concentration in serum (15 mg/l).

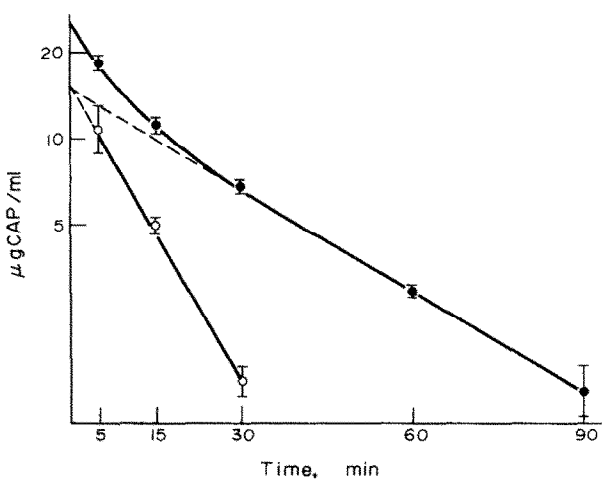

FIG. 1. Exponential decline of the serum concentration of active CAP in normal (●) and phenobarbital pretreated rats (○) after i.v. administration of CAP 30 mg/kg. Half-lifetime: normal 29 min; phenobarbital 8 min. Mean values ± S.E. of five rats for each time.

TABLE 1. CONCENTRATION OF TOTAL CAP, ACTIVE CAP AND CAP GLUCURONIDE IN LIVER AND SERUM OF NORMAL AND PHENOBARBITAL PRETREATED RATS AT DIFFERENT TIMES AFTER CHLORAMPHENICOL (100 mg/kg/per os). ALL VALUES* ARE EXPRESSED AS MICROGRAMS OF CAP equivalents/g LIVER OR ml SERUM

		Time after dose (min)									
		15		30		60		90		120	
		Normal	Pretreated	Normal	Pretreated	Normal	Pretreated	Normal	Pretreated	Normal	Pretreated
Body weight (g)		134 ± 5·90	145 ± 5·4	148 ± 5·8	153 ± 4·3	143 ± 4·8	153 ± 5·3	150 ± 5·4	150 ± 3·1	140 ± 4·1	146 ± 2·4
Liver weight (g) per 100 g body wt		3·7 ± 0·20	4·68 ± 0·18	3·51 ± 0·06	3·96 ± 0·09	3·53 ± 0·13	3·94 ± 0·16	3·44 ± 0·06	4·28 ± 0·13	3·49 ± 0·09	3·88 ± 0·1
Total CAP	Liver	41·9 ± 2·5	22·0 ± 3·0	36·6 ± 1·4	18·0 ± 3·6	27·8 ± 2·5	8·5 ± 1·4	26·1 ± 1·9	5·6 ± 1·6	13·5 ± 1·0	3·0 ± 0·7
	Serum	16·2 ± 1·9	25·7 ± 1·88	24·4 ± 0·7	34·1 ± 3·4	14·8 ± 1·7	15·2 ± 1·14	11·4 ± 1·8	9·7 ± 1·0	8·1 ± 1·2	7·7 ± 0·52
Active CAP	Liver	39·0 ± 2·98	2·7 ± 1·33	32·1 ± 5·29	2·5 ± 0·51	25·0 ± 2·29	1·6 ± 1·1	24·1 ± 3·8	1·0 ± 0·43	1·13 ± 2·5	1·9 ± 0·95
	Serum	11·7 ± 2·49	5·2 ± 0·67	15·5 ± 1·51	3·8 ± 0·60	10·1 ± 1·88	2·2 ± 0·35	7·4 ± 1·25	2·0 ± 0·28	3·0 ± 0·83	1·4 ± 0·07
CAP glucuronide	Liver	2·9	19·3	4·5	15·5	2·8	6·9	2·0	4·6	2·2	1·1
	Serum	4·5	20·5	8·9	30·3	4·7	13·0	4·0	7·7	5·1	6·3

* Mean values of five rats ± S.E.

After oral administration of CAP (100 mg/kg) the peak values in serum were obtained after 15 min in phenobarbital pretreated rats and after 30 min in untreated controls (Fig. 2), but they did not fall exponentially with the half-lifetime measured after i.v. administration, as would be expected if the absorption from the intestine was complete. This indicates that the inflow of CAP into the vascular system continued after its oral administration. The unchanged CAP decreased and the conjugate increased in the serum as well as in the liver in phenobarbital treated rats (Table 1).

By comparing the area under the CAP-concentration–time curve in the blood after i.v. and oral administration (Fig. 2) and assuming that a proportional increase would

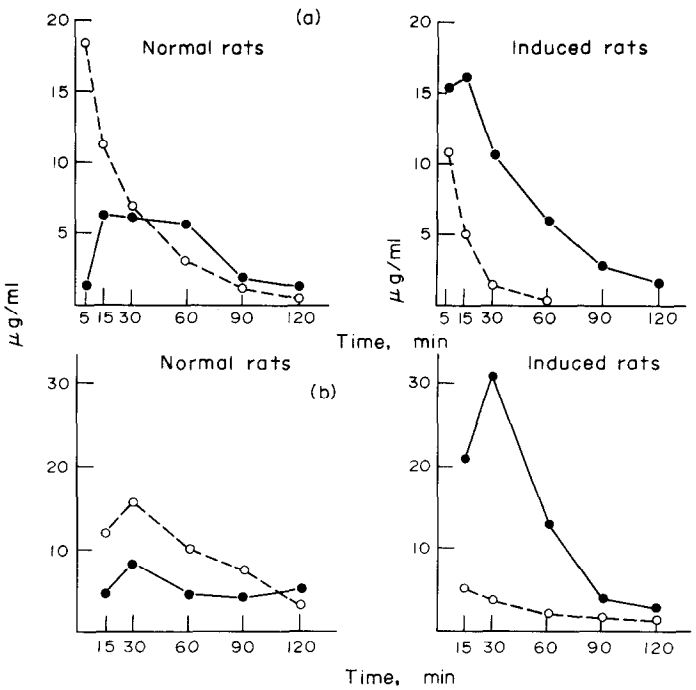

FIG. 2. Serum concentration of active CAP (○) and CAP-glucuronide (●) in normal and phenobarbital pretreated rats at different times after administration of CAP: 30 mg/kg i.v. (a) and 100 mg/kg per os (b).

TABLE 2. AREA BELOW THE CONCENTRATION–TIME CURVE MEASURED IN mg OF THE EXCISED PAPER-WEIGHT

Application of CAP	Pretreatment with phenobarbital	Active CAP	Availability (%)	Ratio without/with phenobarbital	Conjug. CAP
30 mg/kg i.v.	without	351		3·4	225
	with	104			413
Corrected to 100 mg/kg i.v.	without	1170			750
	with	347			1375
100 mg/kg per os	without	550	47	3·5	300
	with	156	44		728

occur if 100 mg/kg were injected i.v. instead of 30 mg/kg it can be calculated (Table 2) that only 45–47 per cent of the orally ingested CAP is available in either phenobarbital-treated or control rats. Less than 50 per cent appeared in the circulation.

DISCUSSION

Low availability of a drug is due either to a slow and insufficient absorption and/or a high metabolism in the liver through which the drug has to pass via the portal vein before gaining access to the vascular system. Undoubtedly the very high rate of conjugation is the main factor for the low availability.

The inflow of unchanged CAP between 90–120 min after ingestion indicates either that CAP is absorbed slowly or that the absorption of CAP continues because of a hepato-intestinal circulation. Excretion of a CAP-conjugate into the bile and reabsorption of the unconjugated CAP after it has been split by bacterial glucuronidase cannot play any significant role because of the high values of the conjugated CAP in the serum after oral administration, indicating that the main portion of conjugated CAP moves from the liver directly into the serum, whereas the part excreted via bile should be eliminated with the faeces without any significant reabsorption. However, chloramphenicol, in spite of its high lipid solubility, is excreted into the bile in small, but significant amounts, as studies in patients with bile fistula have shown.[7] Its concentration in the bile falls much slower than in the plasma. Similar conditions should prevail in rats, suggesting that the delayed absorption of CAP should be due to a hepato-intestinal circulation of the unconjugated CAP.

A comparison of the areas under the time concentration curves of active CAP for normal and pretreated rats discloses that their ratio achieves 3·4 or 3·5 regardless of the route of administration, indicating that about 3·5-fold more CAP is present in the blood of normal rats (Table 2). Correspondingly a much higher output of the conjugate can be found after pretreatment (Table 1). These observations agree completely with the fall of the half-lifetime from 29 to 8 min after pretreatment showing a comparable value, i.e. a 3·6-fold enhanced elimination-rate.

Taking into consideration the much higher concentration of conjugated CAP with concomitant much lower levels of active CAP in the liver of pretreated rats the evidence indicates that an accelerated elimination is due to an increased metabolism in the liver. This conclusion is also justified since only a minor portion of CAP is excreted by the kidney in unchanged form because of its high lipid solubility. Man excretes not more than 10 per cent of active CAP. The rat seems to excrete not more than 25 per cent under the conditions of this experiment, as a comparison of the half-lifetimes of active CAP measured *in vivo* with conjugation rates determined *in vitro* disclosed.[2] Our data agree with studies using the isolated perfused liver from rats as a model.[8] They proved that the clearance of chloramphenicol could be increased 4·7 times if rats were pretreated with phenobarbital.

A three- to four-fold increase of the CAP glucuronidation determined by testing the activity of the transferase in isolated microsomes of rats before and after treatment with phenobarbital provided conclusive evidence that the enhanced elimination rate of CAP from pretreated rats is really caused by an increased conjugation rate.[2]

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