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IN VIVO EFFECT OF CHLORAMPHENICOL AND THIAMPHENICOL ON SOME ENZYMES OF NORMAL MOUSE LIVER

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Abstract—Chloramphenicol a potent inhibitor of bacterial and some mammalian cell protein synthesis, was administered i.p. to a group of mice for 6 consecutive days. Another group of animals was treated similarly with thiamphenicol and a third group served as control. The effects of the two antibiotics on the activity of some liver enzymes; the two pyridoxal 5-phosphate dependent enzymes, kynurenine hydrolase and kynurenine amino-transferase; pyridoxal phosphokinase; β-glucuronidase and acid ribonuclease were determined. Chloramphenicol and thiamphenicol decreased significantly the activities of kynurenine hydrolase, β-glucuronidase and acid ribonuclease and both drugs increased the activity of pyridoxal phosphokinase significantly. Their effect on kynurenine amino-transferase was different, chloramphenicol decreased while thiamphenicol increased the enzyme activity. Results are discussed and possible explanations suggested.

Chloramphenicol (CAP) is a widely used broad spectrum antibiotic. It is a potent inhibitor of protein synthesis of bacterial [1–3] and of mammalian bone marrow cells [4]. The antibiotic is also known to inhibit RNA synthesis [5]. After administration, CAP is widely distributed to virtually all tissues, including the liver where most of it is detoxicated by conjugation with glucuronic acid [6].

Thiamphenicol (TAP) is an analogue of CAP, synthesized by Culter *et al.* [7], has been used clinically as an effective antibacterial agent. CAP and TAP are widely used in Egypt in treatment of Enterica where liver diseases and bilharzial cirrhosis are endemic.

Tryptophan, one of the essential amino acids involved in protein synthesis, has a variety of important metabolic pathways but the one of greatest biological interest, tryptophan–niacin pathway, leads to the formation of nicotinamide. Several enzymes along this route require the participation of pyridoxal 5-phosphate [8, 9].

We planned to study the effects of CAP and TAP on the activities of the two pyridoxal phosphate dependent enzymes, kynurenine hydrolase (EC 3.7.1.3) and kynurenine aminotransferase (EC 2.6.1.7) which metabolize kynurenine to anthranilic and kynurenic acids, respectively [8, 9]. The effects of the two antibiotics on the activities of the liver enzymes, pyridoxal phosphokinase (EC 2.7.1.35) which catalyzes the formation of the coenzyme pyridoxal 5-phosphate [10]; β-glucuronidase (EC 3.2.1.31) which hydrolyzes the conjugated glucuronides of several compounds including drugs; and acid ribonuclease (EC 2.7.7.16) which is related to nucleic acid and protein metabolism [11, 12] were also studied.

MATERIALS AND METHODS

Three groups each consisting of 6 albino mice, weighing 20–30 g, were used. The first group served as control. Mice of group II and III were injected intraperitoneally daily (100 mg/kg) with CAP succinate and TAP respectively, for 6 consecutive days. Controls received equal volumes of the drug solvent. The animals had free access to food and water during the course of treatment. Twenty-four hours after the last injection all the mice were killed by decapitation. The livers were removed and chilled in ice for the assay of enzymes.

The liver homogenates were prepared using a Potter–Elvehjem homogenizer in different solutions and concentrations to measure the activities of the different enzymes studied. Homogenates in 0.7 M phosphate buffer pH 6.8 (25% w/v); in 0.25 M sucrose solution (10% w/v) and in distilled water (2.4% w/v) were prepared and used immediately for determining respectively the activities of pyridoxal phosphokinase; the two kynurenine metabolizing enzymes and both β-glucuronidase and acid ribonuclease.

The activity of pyridoxal phosphokinase was determined by the method of McCormick *et al.* [13]. The incubation medium containing 0.2 mM pyridoxal–HCl, 0.5 mM ATP, 0.01 mM Zn^{2+}, 25% whole liver homogenate in phosphate buffer (0.07 M, pH 6.0) was incubated for one hour at 37°. For determination of the activity of the kynurenine

Table 1. Effect of chloramphenicol and thiamphenicol on the activities of some mouse liver enzymes

	Kynurenine (μg/g liver)				
	Hydrolase	Aminotransferase	Pyridoxal phosphokinase (μg/g liver)	β-glucuronidase (units* × 10^3/g liver)	Acid ribonuclease (units* × 10^3/g liver)
Controls (6)	265 ± 12.0	7.2 ± 0.24	254 ± 10.5	6.9 ± 0.52	0.46 ± 0.04
Chloramphenicol (6)	165 ± 16.1 (38% decrease) P < 0.001	3.5 ± 0.28 (51% decrease) P < 0.001	293 ± 9.9 (15% increase) P < 0.01	2.7 ± 0.28 (60% decrease) P < 0.001	0.22 ± 0.03 (53% decrease) P < 0.001
Thiamphenicol (6)	124 ± 12.1 (53% decrease) P < 0.001	11.3 ± 0.72 (57% increase) P < 0.001	300 ± 3.7 (18% increase) P < 0.01	3.1 ± 0.15 (56% decrease) P < 0.001	0.19 ± 0.03 (58% decrease) P < 0.001

Results presented as mean ± S.E.M.
* Units as defined in the text.

metabolizing enzymes the condition was that of El-Sewedy *et al.* [14]; 5.0 μmoles DL-kynurenine, 30.0 μmoles α-ketoglutarate, 5 mM calcium chloride, 1 M magnesium sulfate, 4.0 μg pyridoxal phosphate and 10% whole liver homogenate in potassium phosphate buffer (0.05 M, pH 7.4). These reagents were incubated at 37° for 2 hr. Kynurenic and anthranilic acids were determined by the methods of Miller *et al.* [15] and Manson and Berg [16], respectively. β-Glucuronidase activity was measured by a modification [17] of the method of Talaly *et al.* [18]. The reaction mixture (0.1 ml substrate 0.01 M, 0.2 ml 2.4% whole tissue homogenate and 0.7 ml McIlvain buffer pH 5.0) was incubated at 37° for 1 hr. One β-glucuronidase unit liberates 1 μg phenolphthalein per hr at 37° [18]. The method of Schucher and Hokin [19] as modified by Veretianer and Straub [20] was used for determining the activity of acid ribonuclease. The incubation condition was 0.2 ml highly polymerized Yeast RNA (2 mg/ml), 0.3 ml 2.4% whole liver homogenate and 0.5 ml acetate buffer (0.1 M, pH 5.0) and the incubation time was 30 min at 37°. The ribonuclease unit was arbitrarily defined according to Sigulem *et al.* [20] as sample absorption at 260 nm—zero time absorption × 1000.

RESULTS

There was no significant difference in the weights of the livers of the CAP and TAP treated mice and those of the untreated ones.

The results of the present study show that CAP significantly ($P < 0.001$) decreased the activity of the two B_6-dependent enzymes; kynurenine hydrolase and kynurenine aminotransferase; as well as the liver β-glucuronidase and the acid ribonuclease (Table 1). The per cent decrease was 38%, 51%, 60% and 53%, respectively. TAP decreased significantly ($P < 0.001$) the activities of β-glucuronidase and acid ribonuclease with per cent inhibition of 56% and 58%, respectively. The effect of TAP on the two kynurenine metabolizing enzyme was different, while it decreased the activity of the B_6-dependent kynurenine hydrolase (53% decreased, $P < 0.001$) it increased the activity of the other B_6-dependent enzyme kynurenine aminotransferase (57% increase, $P < 0.001$). Pyridoxal phosphokinase activity was enhanced by both antibiotics ($P < 0.01$). The per cent increase of CAP was 15% and that of TAP was 18% over the control values.

DISCUSSION

It is evident from the present study that CAP decreases the activity of both the vitamin B_6-dependent enzymes kynurenine hydrolase and kynurenine aminotransferase. The inhibitory effect is more marked for the kynurenine aminotransferase than for the kynurenine hydrolase (51%, 38%, respectively). The kynurenine aminotransferase is strictly mitochondrial whereas the kynurenine hydrolase is present in the supernatant fraction [22]. CAP was found to be an inhibitor of another mitochondrial enzyme, liver monoamine oxidase [23]. In contrast to the above similarity between the effect of CAP on the two kynurenine metabolizing enzymes, there is a distinct difference between the effect of TAP on both enzymes. While TAP decreased kynurenine hydrolase activity (53%) it increased the activity of kynurenine aminotransferase to nearly the same extent (57%). This is not an extraordinary observation since similar difference in response of these two enzymes to several drugs was reported [8, 20]. TAP seems to block kynurenine metabolism via anthranilic acid formation to direct the accumulated kynurenine to be metabolized to kynurenic acid.

At present, relatively little is known about the factors regulating the activity of hepatic pyridoxal phosphokinase. The present study shows that both CAP and TAP significantly increased its activity. A recent publication by some of us showed that anthranilic acid and kynurenic acid had no effect on the activity of liver pyridoxal phosphokinase *in vitro* [25]. The finding that CAP and TAP did not decrease the activity of hepatic pyridoxal phosphokinase, shows that a deficiency of the coenzyme pyridoxal 5-phosphate could not explain the inhibitory effects of these drugs encountered on the kynurenine metabolizing enzymes.

CAP and TAP decreased significantly the activity of the two liver lysosomal enzymes studied β-glucuronidase and acid ribonuclease. Both drugs are able to penetrate the lipid layers of membranes and to gain access to the lysosomal proteins [26]. The drugs or their metabolites may inhibit the activity of these two enzymes by impairment of the protein enzymes. The role of the ribonuclease enzyme is still unknown, however it has been proposed that an inhibitor ribonuclease system is a part of a complex regulating mechanism that evidently controls the production of cellular RNA, through influencing the

rate of RNA degradation [27]. The concomitant protein synthesis and growth are consequently affected. Therefore, CAP and TAP may affect protein synthesis through its inhibitory effect on liver ribonuclease enzyme.

It could be concluded from the present study that the administration of CAP and TAP induces abnormal kynurenine metabolism. This could be attributed to a functional B_6-deficiency which may be due to the interaction of these drugs or their metabolites with the coenzyme pyridoxal 5-phosphate.

However, it is still possible that an impairment of the kynurenine hydrolase and kynurenine aminotransferase apoenzymes, as well as β-glucuronidase and acid ribonuclease protein enzymes, may be responsible at least in part for the observed decreased activity of these enzymes caused by CAP and TAP.

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