THE EFFECT OF CHLORAMPHENICOL TREATMENT ON FERROCHELATASE ACTIVITY IN DOGS

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Summary. Chloramphenicol fed to dogs at a dose of 100 mg/kg/day resulted in a decrease in bone marrow ferrochelatase activity to 5-35% of control levels. Chloramphenicol in concentrations of 25 and 50 μg/ml had no effect on ferrochelatase activity in vitro.

Chloramphenicol (CAP) is a potent inhibitor of mammalian mitochondrial protein synthesis (1 - 4). In further studies it was shown that this metabolic action of CAP affects primarily the structural or membranous proteins (5 - 7).

Recent work demonstrating that the enzyme ferrochelatase is associated with the inner mitochondrial membrane (8, 9) raises the important question of whether the synthesis of this enzyme is inhibited by CAP. Inhibition of ferrochelatase synthesis could represent a major biochemical mechanism underlying reversible erythroid suppression from the drug (10).

In the following study we have examined the effect of CAP treatment on the activity of bone marrow ferrochelatase in dogs.

Materials and Methods. Seven adult female mongrel dogs were purchased from Pel-Freeze Laboratories (Arkansas, U.S.A.). Protoporphyrin was obtained from Calbiochem. $^{59}FeCl_3$ was purchased from New England Nuclear. All dogs were maintained on Purina dog meal. Water was given ad lib.

The animals were divided into three groups as follows: Group 1 - dogs were given CAP (Chloromycetin®, Parke Davis & Co., 250 mg. capsules) orally at a dose of 100 mg/kg/day (serum free CAP levels on this regimen ranged from 11 to 16 μg/ml); groups 2 and 3 dogs served as controls and received tetracycline (Steclin®, E. R. Squibb & Sons, Inc. 250 mg. capsules) and sucrose respectively in the same dose. Three weeks, during which no experimentation was done on the animals, was allowed for an adjustment period.

Approximately 40 cc. of marrow was aspirated from the iliac crest, by multiple puncture, using a University of Illinois type sternum needle (Scientific Products) under light sodium pentobarbital anesthesia. The marrow was collected in heparin and dispersed by gentle aspiration into and ejection from a syringe. All glassware was siliconized and, unless otherwise indicated, all operations were carried out at 0-4°.

The marrow was centrifuged for 5 min. at 200 x g. followed by 750 x g. for 10 min. The resulting upper "buffy coat" and top 1/4 of the red blood cell residue were aspirated and suspended in physiologic saline. After dispersion, a nucleated cell count was done, and the suspensions were adjusted to approximately the same counts by appropriate dilution. The final count in different experiments ranged from 80,000 to 110,000 nucleated cells/mm^3 in a volume of about 15 ml. Stained smears were then prepared and differential cell counts performed to determine the percentage of nucleated erythroid cells.

The cells were washed twice in 15 ml. of physiologic saline, resuspended in 15 ml. of saline and broken in the French Pressure Cell at a pressure of 2500 p.s.i. Mitochondria were prepared from this homogenate according to the procedure of Hogeboom (11) using the centrifugation speeds

recommended by Meyers and Slater (12). The final mitochondrial pellet was suspended in 0.1 M phosphate buffer, pH 7.4. The protein concentration of this mitochondrial pellet was assayed by the method of Lowry (13).

Ferrochelatase activity was assayed as follows. The reaction mixture consisted of phosphate buffer, pH 7.4, 75 mM; reduced glutathione, 10 mM; protoporphyrin, 30 μM; $^{59}FeCl_3$, 2.5-3.0 μc (specific activity 10-16 mc/mg Fe) and mitochondria 3 to 5 mg. in a final volume of 1.56 ml. The incubations were carried out in duplicate or triplicate in 25 ml. flasks under air in a Dubnoff Metabolic Shaker at 37° for 3 hrs. The reaction was stopped by rapid cooling to 0°.

The contents of the incubation flasks were centrifuged and a portion of the supernatant was spotted on Whatman No. 3 chromatography paper and developed in a 1:3 (V/V) pyridine-methanol system (14). Under these conditions non-heme iron remained at the origin and the heme traveled at a R_f of 0.80. The strips were counted in a Vanguard Model 880 Automatic Chromatogram Scanner attached to a Nuclear Chicago Integrator. The net counts used in calculating the results were in all cases at least 3-8 times the background. Duplicate and triplicate samples did not vary more than 10%. The results were expressed as picomoles ^{59}Fe incorporated into heme per 10^8 nucleated red cells or per mg. mitochondrial protein per 3 hr. at 37°

Results. Table I shows the results of the different ferrochelatase assays done at the intervals indicated. CAP reduced ferrochelatase activity under the experimental conditions used to between 5% and 35% of the control values (e.g. those animals receiving tetracycline or sucrose). Also from Table I it can be seen that essentially maximum depression of ferrochelatase activity occurred sometime during the first 3 weeks of feeding the drug since the experiments done at 35 and 56 days show no further

TABLE I

The in vivo effect of CAP on ferrochelatase activity in dog bone marrow mitochondria.

Days of Therapy	FERROCHELATASE ACTIVITY*						
	Controls				Chloramphenicol		
	Sucrose		Tetracycline				
	Dog 1	2	3	4	5	6	7
0 (control)	69.4	68.7	58.5	117.1	53.0	67.6	48.2
21	59.6	56.4	48.2	58.3	16.6	9.3	15.7
35	50.0	-	-	-	-	7.4	$\langle$ 1.5
40	-	36.8	45.6	49.2	17.4	-	-
56	84.5	60.0	46.4	52.6	$\langle$ 1.5	17.7	3.9

* Values represent picomoles ^{59}Fe incorporated into heme/10^8 nucleated red blood cells/3 hrs. at 37° and are the averages of duplicate or triplicate assays. Dashes indicate that activity was not determined. Reaction mixture as described in Materials and Methods.

TABLE II

The in vitro effect of chloramphenicol on ferrochelatase activity in bone marrow mitochondria.

Final Chloramphenicol Concentration, μg/ml	Ferrochelatase Activity*
0	9.7
25	8.3
50	10.8

*Values represent picomoles ^{59}Fe incorporated into heme/mg mitochondrial protein/3 hrs. at 37° and are the averages of duplicate assays. Reaction mixture as described in Materials and Methods.

decrease in activity. That the results of the in vivo effects of CAP depicted in Table I were not due to some direct effect of CAP on ferrochelatase (e.g. binding to the enzyme) has been ruled out by the in vitro experiments (Table II) where CAP concentrations up to 50 μg/ml did not affect activity.

Discussion. These data show that in vivo, CAP reduced ferrochelatase activity when compared to animals fed tetracycline or sucrose, while having no effect in the in vitro system. Expressing the results of Table I on the basis of picomoles iron incorporated per mg. mitochondrial protein (as in Table II), the control values ranged from 9.2 to 17.2 picomoles iron incorporated while the experimental values ranged from 1 to 4.2 picomoles iron incorporated.

The mechanism of suppression of ferrochelatase activity in dogs receiving CAP in uncertain. A reasonable explanation of the results presented here, in light of previous work (5 - 7) is that CAP inhibited the synthesis of ferrochelatase in mitochondria. However, other possibilities must be considered. A direct effect of CAP on ferrochelatase activity was ruled out by our experiments (Table II). Another possible explanation of the data would be that CAP affects some enzyme or enzyme system which in turn controls ferrochelatase.

Chloramphenicol is known to suppress erythropoiesis when given in large doses (10). The early manifestations of this toxic effect consist of a rise in serum iron, increase in the saturation of iron binding globulin, and a decrease in plasma iron clearance and utilization (15). The profound effect of chloramphenicol on ferrochelatase activity observed in this study offers a reasonable biochemical explanation for these changes and could account for the apparent vulnerability of nucleated red blood cells to this drug.

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