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INHIBITION OF MICROSOMAL DRUG METABOLIC PATHWAYS BY CHLORAMPHENICOL*

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Abstract—Chloramphenicol can prolong the hypnotic action of hexobarbital in mice and also slow its rate of biotransformation. Added *in vitro*, chloramphenicol inhibits a number of pathways for drug metabolism present in liver microsomes. Data presented indicate that this inhibition by chloramphenicol may be noncompetitive and irreversible with acetanilid and hexobarbital as substrates. These same pathways are also blocked by SKF 525-A and certain other compounds.

CHLORAMPHENICOL can prolong the duration of action of hexobarbital in rats and mice. This observation prompted an investigation of the mechanism by which chloramphenicol produces this effect. The duration of action of many drugs may be prolonged by compounds such as β-diethylaminoethyldiphenylpropylacetate (SKF 525-A) which act as inhibitors of drug metabolism.[1] This paper presents evidence that chloramphenicol resembles SKF 525-A in several actions including this ability to inhibit hepatic microsomal drug metabolism.

METHODS

Materials

Chloramphenicol (for intramuscular injection) and chloramphenicol sodium succinate (for intravenous injection) were used. SKF 525-A was obtained through the courtesy of Smith, Kline, & French Laboratories.

In vitro-*experiments*

The metabolic reactions studied were the side-chain oxidation of hexobarbital, the O-dealkylation of codeine, the N-dealkylation of aminopyrine, and the hydroxylation of the aromatic ring of acetanilid. The rate of metabolism of hexobarbital was determined by measuring the rate of its disappearance. The rates of appearance of a metabolite (morphine, 4-aminoantipyrine, and N-acetyl-*p*-aminophenol, respectively) were measured to follow the metabolism of codeine, aminopyrine, and acetanilid. 4-Aminoantipyrine was measured as described by Brodie and Axelrod.[2] Hexobarbital was determined by the method of Cooper and Brodie.[3] The method of Mitoma *et al.*[4] was used to assay the hydroxylation of acetanilid. Morphine was extracted from tissue with chloroform and determined by a phenol reagent.[5]

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The incubation conditions, cofactors added, and their concentrations were the same as those used by McLuen and Fouts.[6] Male white mice (25–30 g) were killed and their livers chilled after removal of the gall bladders. The livers from 40–50 mice were pooled. The tissue was homogenized in 2 vols of 1·15 per cent KCl by means of a glass homogenizer with a plastic pestle. A supernatant fraction containing microsomes and the soluble fraction was prepared by centrifuging the homogenate at 9000 × *g* for 20 min at 4 °C.

In vivo-*studies*

Sleeping times after administration of hexobarbital sodium to animals pretreated with saline were compared with those in chloramphenicol-pretreated animals. Hexobarbital sodium was injected as a 1 per cent solution intraperitoneally (i.p.) at a dose of 100 mg/kg, and the time in min from the loss of the righting reflex until its recovery was recorded as "sleeping time". Chloramphenicol was given intravenously (i.v.), i.p., or orally.

Whole-body levels of hexobarbital in normal mice and in those pretreated with chloramphenicol were determined at various times after its administration. Animals were killed, and each animal was homogenized in 1·15 per cent KCl in a Waring Blendor. The total volume was 100 ml. An aliquot of the homogenate was taken and its content of hexobarbital determined.

Statistical calculations used are described by Snedecor.[7] P is the probability that no difference other than that caused by random error exists between control and treated animals as determined by Student's "t" test. The level of significance used is $P < 0{\cdot}05$.

TABLE 1. HEXOBARBITAL SLEEPING TIMES AFTER CHLORAMPHENICOL AND SKF 525-A

Pretreatment*	Sleeping time†
Saline	21 ± 5 (20)‡
Chloramphenicol, 6·25 mg/kg	39 ± 9 (10)
Chloramphenicol, 12·5 mg/kg	52 ± 18 (20)
Chloramphenicol, 25·0 mg/kg	84 ± 29 (20)
Chloramphenicol, 50·0 mg/kg	98 ± 27 (20)
SKF 525-A, 12·5 mg/kg	109 ± 23 (10)
SKF 525-A, 25·0 mg/kg	141 ± 62 (20)

* Animals received chloramphenicol or SKF 525-A (i.p.) 45 min prior to injection (i.p.) of hexobarbital.

† Loss of righting reflex after injection of hexobarbital, 100 mg/kg

‡ Sleeping times expressed as average plus or minus standard deviation. Number of animals per group is indicated in parentheses.

RESULTS

Effect of chloramphenicol and SKF 525-A on duration of action of hexobarbital in mice

Mice pretreated with chloramphenicol sleep longer after hexobarbital administration than do mice pretreated with saline (Table 1). The duration of action of hexobarbital increases as the dose of chloramphenicol increases. Significant effects of chloramphenicol are hard to demonstrate below the dose-level of 6·25 mg/kg (Table 1). Neither the form of chloramphenicol (free base vs. succinate salt) nor the route of

administration (i.v., i.p. or oral) that was used seem to affect markedly this action of the antibiotic. Chloramphenicol was less potent than was SKF 525-A (Table 1).

Duration of effect of chloramphenicol in vivo

Sleeping times were determined when chloramphenicol was given at different times before and after the hexobarbital. The antibiotic had its greatest effect on sleeping time when administered 45 min before injection of the barbiturate (Table 2). The

TABLE 2. EFFECT OF TIME OF ADMINISTRATION OF CHLORAMPHENICOL ON ITS PROLONGATION OF HEXOBARBITAL SLEEPING TIME

Time of injection of chloramphenicol* relative to hexobarbital (hxb.) administration	Sleeping time (min)	P†
Control‡	19 ± 3§	
10 min after hxb.	27 ± 11	0·4–0·2
Simultaneously with hxb.	31 ± 13	0·025–0·010
45 min before hxb.	62 ± 7	<0·001
90 min before hxb.	48 ± 14	<0·001
3 hr before hxb.	42 ± 13	<0·001
6 hr before hxb.	38 ± 11	<0·001
12 hr before hxb.	20 ± 4	>0·5
24 hr before hxb.	19 ± 3	>0·5
48 hr before hxb.	19 ± 3	>0·5
72 hr before hxb.	17 ± 4	0·4–0·2
96 hr before hxb.	20 ± 5	>0·5

* Chloramphenicol succinate injected 25 mg/kg i.p.

† Comparing control animals with chloramphenicol-treated groups.

‡ All groups had 10 animals.

§ Values are averages plus or minus standard deviation.

sleeping times of animals pretreated with chloramphenicol at times from 12–96 hr before the administration of hexobarbital did not differ significantly ($P \geq 0{\cdot}05$) from those of control animals. Fujimoto and Serrone[8] have reported that various inhibitors of hexobarbital metabolism can cause a shortened "hexobarbital sleeping time" in mice when these inhibitors are injected 24–48 hr before the barbiturate.

Effect of chloramphenicol on the rate of biotransformation of hexobarbital

The rates of biotransformation of hexobarbital in control mice were compared with those in mice that were pretreated with 50 mg of chloramphenicol succinate/kg. Forty-five min after treatment i.p. with either saline or chloramphenicol, the mice were injected i.p. with 150 mg of hexobarbital sodium/kg. For both pretreated and control animals two hexobarbital determinations were made. For the first determination, animals from each group were killed by a blow on the head 20 min after the injection of hexobarbital. For the second determination in each series the mice were killed upon awakening. Whole-body levels of hexobarbital in each of these groups of animals were determined as described under Methods.

The whole-body levels of hexobarbital in the chloramphenicol-treated group of animals were significantly higher 20 min after hexobarbital administration than in the group of animals receiving a saline pretreatment (Table 3). This indicated that

chloramphenicol increased the biological half-life of the barbiturate. However, the whole-body levels of barbiturate at the time of recovery of the righting reflex in animals pretreated with chloramphenicol were not significantly different ($P \geq 0{\cdot}05$) from the amount of hexobarbital in animals which received only saline. Evidence against a direct central depressant action of chloramphenicol was obtained by injecting the

TABLE 3. EFFECT OF CHLORAMPHENICOL ON WHOLE-BODY CONCENTRATION OF HEXOBARBITAL IN MICE

Time of sacrifice	Control	Chloramphenicol-treated	P*
20 min after injection†	69 ± 20 mg/kg‡	99 ± 9 mg/kg	0·01
Upon awakening	35 ± 4 mg/kg	45 ± 11 mg/kg	0·1

* Control animals compared with chloramphenicol-treated group.

† Animals were injected with hexobarbital, 150 mg/kg, i.p.

‡ Average ± standard deviation mg/kg determined as whole-body level. All groups have 6 animals.

antibiotic (i.p.) in doses of 50–100 mg/kg into mice which had just recovered their righting reflex after hexobarbital. None of the animals so treated returned to sleep (lost their righting reflex), although they recovered from the post-hypnotic effects of the barbiturate more slowly than did animals that had not received an additional dose of chloramphenicol.

TABLE 4. CONCENTRATION OF CHLORAMPHENICOL* OR SKF 525-A PRODUCING 50 PER CENT INHIBITION OF VARIOUS DRUG METABOLIC PATHWAYS *in vitro*

Substrate	Product	Concentration required for 50% inhibition†	
		Chloramphenicol	SKF 525-A
Hexobarbital	Ketohexobarbital	19 ± 20 (5)‡	0·5§
Acetanilid	N-acetyl-*p*-aminophenol	69 ± 34 (7)	5
Codeine	Morphine	87 ± 12 (6)	8
Aminopyrine	4-Aminoantipyrine	50 ± 8 (4)	5

* Chloramphenicol was used as the sodium succinate salt.

† Concentrations of inhibitors are M × 10^{-5}.

‡ Values are averages plus or minus standard deviation. The figures in parentheses represent the number of determinations (separate experiments).

§ Values are the average of two determinations.

Effects of chloramphenicol on a variety of drug pathways in vitro

The inhibitory effects of chloramphenicol on metabolic pathways of several drugs were compared in a supernatant fraction (9000 × *g*) of mouse liver. A range of concentrations of chloramphenicol was used, varying from 5×10^{-3} to 10^{-6} M. The concentrations producing 50 per cent inhibition (I_{50}) (Table 4) were interpolated from plots of per cent inhibition against the logarithm of chloramphenicol concentration. Concentrations of SKF 525-A that produce 50 per cent inhibition of the rate of

metabolism of the same group of drugs are also presented in Table 4. These I_{50} concentrations for chloramphenicol varied from one experiment to another, as shown by the large standard deviations.

Mechanism of inhibition

In order to determine whether the inhibition *in vitro* of the drug-metabolizing enzymes was competitive, the reciprocal of the velocity of metabolism was plotted against the reciprocal of the substrate concentration. For each concentration of the antibiotic the best-fitting straight line was drawn. These plots, when interpreted as described by Dixon and Webb,[9] indicated that the inhibition by chloramphenicol was of a noncompetitive nature with both acetanilid and hexobarbital as substrates. Fig. 1 shows such a plot with acetanilid as substrate.

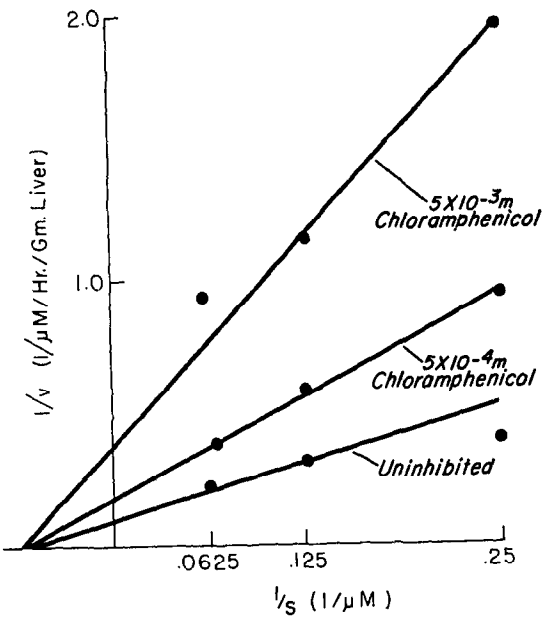

FIG. 1. Non-competitive inhibition by chloramphenicol of acetanilid metabolism in 9000 g supernatant fraction. Enzyme preparation and cofactors, as described in Methods, were incubated with varying concentrations of acetanilid (S) and chloramphenicol for 1 hr and assayed for N-acetyl-*p*-aminophenol produced (V).

To determine whether inhibition by chloramphenicol was reversible, 9000 × *g* supernatant, and 9000 × *g* supernatant plus chloramphenicol, were dialysed in a rocking dialyser against cold, running, distilled water for 3 or 24 hr after which time the rates of metabolism of hexobarbital, acetanilid, and aminopyrine were determined. When the per cent inhibition of metabolism in the dialysed samples was compared with the per cent inhibition of metabolism in the undialysed samples there was no significant difference. These results indicated that the inhibition produced by chloramphenicol was not reversed by dialysis.

DISCUSSION

The results reported in this paper indicate that chloramphenicol can inhibit the metabolic transformation of a number of drugs. Chloramphenicol can also prolong the duration of action of hexobarbital in mice. This latter effect is unlikely to be caused by a central action of the antibiotic because: (a) mice given chloramphenicol upon regaining their righting reflex after treatment with hexobarbital do not return to sleep; (b) determinations of whole-body levels of hexobarbital in mice indicate that hexobarbital disappears more slowly in chloramphenicol-pretreated animals than in control animals; (c) both the control and chloramphenicol-pretreated animal, however, have the same whole-body levels of hexobarbital upon recovery of the righting reflex. Thus, chloramphenicol is not a "potentiator" in the commonly used sense of the word, since it does not cause subhypnotic doses of hexobarbital to become effective. It would be more correct to classify chloramphenicol as a prolonging agent along with such drugs as SKF 525-A, Lilly 18947 (2,4,-dichloro-6-phenyl-phenoxyethyl diethylamine)[10] and iproniazid.[11] The effect of chloramphenicol on several other enzyme systems has been reviewed by Hunter.[12]

Studies on the mechanism of inhibition indicate that the action of chloramphenicol on drug enzymes may be both noncompetitive and irreversible. Studies of the nature of enzyme inhibition by the methods of Lineweaver and Burk[13] or Dixon and Webb[9] should be interpreted with care; Michaelis-Menten equations assume a reversible combination of enzyme and inhibitor.

The apparent inability of chloramphenicol to cause a shortened hexobarbital sleeping time 12–96 hr after administration of chloramphenicol is in contrast to most other enzyme-inhibiting drugs that have been studied.[8]

Possible clinical effects caused by the inhibitory action of chloramphenicol on drug metabolism might include a greater response to a drug in patients also receiving massive doses of chloramphenicol.

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