PROTECTIVE EFFECT OF CHLORAMPHENICOL AND DEXTRAMYCIN AGAINST THE ADRENOCORTICOLYTIC ACTION OF 7,12-DIMETHYLBENZ(a)ANTHRACENE

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The effect of chloramphenicol (CA) and dextramycin (DM) on the adrenocorticolytic action of 7,12-dimethylbenz(a)anthracene (DMBA) was studied in rats. Administration of CA in doses of 0.1-1.0 mg/g and of DM in doses of 0.05-1.0 mg/g 1.5 h before the carcinogen was shown to prevent the development of adrenal necrosis completely. If smaller doses of CA and DM were given, and also if they were given 48 h before the carcinogen or 1.5 h after it, the protective effect was partially preserved. If CA and DM were given 3, 6, or 24 h after DMBA the toxic action of the carcinogen was unchanged.

KEY WORDS: *chloramphenicol; dextramycin; 7,12-dimethylbenz(a)anthracene; protection against adrenocorticolytic action.*

A previous investigation [1] showed that the antibiotic chloramphenicol (CA) and its optical isomer dextramycin (DM) prevent the adrenocorticolytic action of 7,12-dimethylbenz-(a)anthracene (DMBA) in rats.

To study the mechanism of the antitoxic action of these compounds, an important step is to discover the minimal protective doses, which evidently also ought to give the minimal side effects, and also to discover how the time of administration of the compounds (before or after the carcinogen) influences their protective effect. The investigation described below was carried out to study these problems.

EXPERIMENTAL METHOD

Experiments were carried out on female noninbred rats weighing 120-150 g. To induce necrosis of the adrenals a solution of DMBA (Ferak, Berlin) in sunflower oil was given by gastric tube in a dose of 20-30 mg per rat. CA and DM (the latter synthesized by Ya. S. Karpman, Akrikhin Factory) also were given by gastric tube in doses of 0.01-1 mg/g at different times before or after the carcinogen. Weighed samples of CA or DM were mixed with starch, covered with boiling water, and stirred vigorously until a homogeneous suspension was formed. The quantity of starch in the suspension was 2%.

The animals were studied 48-72 h after administration of DMBA. The adrenals were taken from all the rats (killed and dying) and fixed in 10% formalin; histological sections were then cut by the standard method, stained with hematoxylin–eosin, and examined for the presence of necrosis. Necrosis of the cortical layer of the adrenals, sometimes with extensive hemorrhages in the areas of necrosis, was observed microscopically after administration of DMBA. In most cases total destruction of all layers of the cortex was observed and the zone of necrosis was structureless, with fragments of nuclei. Even where there were clearly defined necrotic changes in the adrenal cortex, it must be emphasized that the medulla appeared intact.

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TABLE 1. Effect of Various Doses of CA and CM on the Adrenocorticolytic Action of DMBA

Experiment No.	Treatment: DMBA, mg per rat	Treatment: CA, mg/g	Treatment: DM, mg/g	No. of animals: dying	No. of animals: with necrosis
1	24	—	—	3/5	5/5
	24	1	—	0/5	0/5
2	20	—	—	4/5	5/5
	20	0,1	—	0/5	0/5
	20	0,05	—	1/5	3/5
	20	0,01	—	3/5	3/5
	20	—	1	0/5	0/5
	20	—	0,1	0/5	0/5
	20	—	0,05	0/5	0/4
3	30	—	—	4/5	5/5
	30	0,05	—	0/5	1/5
	30	0,01	—	3/5	5/5
	30	—	0,05	0/5	0/5
	30	—	0,01	0/5	4/5*
4	30	—	—	1/5	5/5
	30	0,005	—	1/5	5/5
	30	—	0,01	0/5	2/5*
	30	—	0,005	0/5	5/5
	30	—	0,001	0/5	5/5

TABLE 2. Effect of CA and CM on Dose of 1 mg/g on the Adrenocorticolytic Action of DMBA Depending on its Time of Administration (before or after the carcinogen)

Experiment No.	DMBA, mg per rat	Treatment: CA after DMBA 1½ h	CA 3 h	CA 6 h	CA 24 h	DM 1½ h	DM 3 h	DM 6 h	DM 24 h	Number of animals: dying	Number of animals: with necrosis
1	24	—	—	—	—	—	—	—	—	3/5	5/5
	24	+	—	—	—	—	—	—	—	0/5	0/5
2	20	—	—	—	—	—	—	—	—	3/5	3/5
	20	+	—	—	—	—	—	—	—	0/5	0/5
	20	—	—	—	+	—	—	—	—	0/5	4/5
3	20	—	—	—	—	—	—	—	—	4/5	5/5
	20	—	—	—	—	+	—	—	—	0/5	0/5
4	30	—	—	—	—	—	—	—	—	1/5	5/5
	30	—	—	+	—	—	—	—	—	1/5	5/5
	30	—	—	—	+	—	—	—	—	1/5	5/5
	30	—	—	—	—	—	+	—	—	1/5	4/4*
	30	—	—	—	—	—	—	+	—	2/5	5/5
	30	—	—	—	—	—	—	—	+	2/5	5/5
5	30	—	—	—	—	—	—	—	—	3/5	5/5
	30	—	+	—	—	—	—	—	—	1/5	4/5
	30	—	—	+	—	—	—	—	—	4/5	5/5
	30	—	—	—	—	+	—	—	—	0/5	2/5
	30	—	—	—	—	—	+	—	—	3/5	4/5
	30	—	—	—	—	—	—	+	—	2/5	5/5
	30	—	—	—	—	—	—	—	+	4/5	5/5
6		CA before DMBA: 48 h		CA before DMBA: 72 h							
	24	—		—						3/5	5/5
	24	+		—						0/5	2/5
	24	—		+						0/5	4/5

Legend to Tables 1 and 2. 1) Numerator gives number of dying animals or number of animals with adrenal necrosis; denominator gives number of animals in group. 2) In experiments Nos. 1, 4, and 6 the animals were killed 48 h and in experiments Nos. 2, 3, and 5 72 h after administration of DMBA. 3) Asterisk denotes discovery of foci of commencing necrosis on microscopic examination.

EXPERIMENTAL RESULTS AND DISCUSSION

The effect of CA and DM on the adrenocorticolytic action of DMBA is illustrated by the results given in Table 1. After administration of DMBA in a dose of 20-30 mg per rat, areas of necrosis developed in the adrenals of 100% of the animals in all the experiments; 48 h after administration of DMBA (experiments Nos. 1 and 4), 4 of the 10 rats, and 72 h after DMBA (experiments Nos. 2 and 3), 8 of the 10 rats died. If, however, 1.5-2 h before the DMBA the animals were given CA in a dose of 1 or 0.1 mg/g, death of the animals and the development of adrenal necrosis were completely prevented. In a dose of 0.05 mg/g (experiments Nos. 2 and 3) CA still gave a protective effect but it was weaker. DMBA caused the death of 1 of the 10 rats and necrosis of the adrenals developed in 4 of the 10 animals. In a dose of 0.01 and 0.005 mg/g CA did not affect the toxic action of DMBA: The carcinogen caused the development of adrenal necrosis in 80 or 100% of the animals, respectively.

DM had a complete protective effect in doses of between 1 and 0.05 mg/g and prevented death and the development of adrenal necrosis in all the animals. In a dose of 0.01 mg/g the protective effect of DM was much weaker, but even in this case the number of dying animals was reduced and only 60% of the rats developed adrenal necrosis; moreover, only discrete foci of necrosis were discovered in them. In smaller doses (0.005 and 0.001 mg/g) DM did not affect the toxic action of DMBA and 100% of the animals developed adrenal necrosis.

The results of the experiments in which the change in the toxic action of DMBA was studied in relation to the time of administration of CA and DM are given in Table 2. Just as in the previous experiments, DMBA in a dose of 20-30 mg per rat caused the development of adrenal necrosis in 100% of the animals. If CA or DM was given to the animals in a dose of 1 mg/g 1.5 h after DMBA, the toxic action of the carcinogen was virtually totally abolished: All the rats survived and only in one experiment (No. 5) did adrenal necrosis develop in 2 of the 5 rats after administration of DM. If CA was given 48 h before the carcinogen, the protective effect was preserved only partially. Finally, if CA and DM were given 3, 6, or 24 h after the carcinogen or 72 h before it, the toxic action of the DMBA was unchanged and the number of dying animals and of animals with adrenal necrosis was approximately the same as when DMBA was given alone.

The protective effect of CA was thus completely preserved when the dose was reduced from 1 to 0.01 mg/g and partially preserved when reduced to 0.05 mg/g. The effect of DM on the toxic action of DMBA was more marked and the protective effect of this substance was completely preserved when its dose was reduced to 0.05 mg/g. This is in agreement with previous observations in which DM reduced the carcinogenic action of urethane somewhat more strongly than CA (unpublished data).

According to data in the literature, carcinogenic polycyclic hydrocarbons, including DMBA, belong to the class of what are called procarcinogens, which become toxic and carcinogenic for cells only after metabolic conversions in the microsomal system of multipurpose oxidases [4]. By changing the activity of this enzyme system by means of inhibitors or inducers, the fate of the "procarcinogens" can be substantially modified [7-9] and, in particular, the adrenocorticolytic action of DMBA can be reduced [2, 3, 6]. As regards CA and DM, only their inhibitory action of DMBA metabolism evidently needs to be considered. Stimulation of metabolism through the action of inducers, due to the synthesis of fresh molecules of the enzymes, requires time and the protective effect is usually manifested only when the carcinogen is given at least 24-72 h after the inducer [5]. In the present experiments both compounds completely prevented the development of adrenal necrosis in the animals when given not only 1.5 h before, but also 1.5 h after the carcinogen. Moreover, the protective effect of CA was preserved only partially when it was given 48 h before DMBA and it was completely absent when CA was given 72 h before the carcinogen. The writers previously observed a similar decrease in the protective effect of CA and DM against the adrenocorticolytic action of 7-acetoxymethyl-12-methylbenz(a)anthracene [1]. In addition, since the effect of the same substance can be qualitatively altered by varying the substrate on which it acts, before a final solution is reached for the problem of the possible role of microsomal enzymes in the protective effect of CA and DM, the effect of these compounds must be studied on DMBA metabolism not only in the liver, but also in the adrenals, which are sensitive to the toxic action of this carcinogen.

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