CHLORAMPHENICOL AND DEXTRAMYCIN AS INHIBITORS OF MAMMARY GLAND CARCINOGENESIS INDUCED BY 7,12-DIMETHYLBENZ(a)ANTHRACENE

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Experiments on noninbred female rats showed that chloramphenicol and its optical isomer dextramycin reduce the carcinogenic action of 7,12-dimethylbenz(a)anthracene on the mammary gland. The protective action was expressed as a decrease in the percentage of animals with tumors at all times of observations, an increase in the length of survival of the rats and, in the case of dextramycin, an increase in the latent period of onset of the tumors.

KEY WORDS: chloramphenicol; dextramycin; 7,12-dimethylbenz(a)anthracene; carcinogenesis; mammary gland.

The inhibitory effect of chloramphenicol (CAP) and dextramycin (DMC) on the development of tumors of the liver and lungs has been the subject of many investigations [5-8, 12].

The object of this study was to examine the effect of these compounds on the development of mammary gland tumors induced by 7,12-dimethylbenz(a)anthracene (DMBA).

METHODS

DMBA (Fluka AG, Buchs SG), dissolved in sunflower oil, was administered perorally through a tube to noninbred female rats weighing 120-150 g in a total dose of 10 mg per rat. Some of the rats also were given CAP or DMC per os 1.5-2 h before administration of the carcinogen, in a dose of 1 mg/kg, which completely protects animals against the adrenocorticolytic action of DMBA [1, 3]. Groups of intact animals and also rats receiving CAP or DMC only were used as controls to all the experiments.

The animals were palpated every 2 weeks and the appearance of tumors was noted. Mammary gland tumors in all animals were studied histologically. The results were subjected to statistical analysis by Wilcoxon's two-sample test [2].

RESULTS

In the first experiment 10 mg DMBA was given as a single dose. This dose proved to be toxic: On the 4th-6th day 16 of the 37 rats receiving the carcinogen alone died with necrosis in the adrenal cortex. Later, these rats were disregarded when animals with tumors were counted. The first tumor in this group appeared after 10 weeks (Fig. 1A) in one (4.8%) of 21 rats. By the end of the experiment (24 weeks) tumors had developed in 28.6% of animals.

In rats receiving CAP and DMBA tumors appeared 2 weeks later, i.e., after 12 weeks, in three (12.5%) of the 24 rats simultaneously. By the end of the experiment tumors had developed in 38% of the rats.

In the group of animals receiving DMC and DMBA, the first tumor also appeared by the 12th week (in one of 26 rats, 3.8%). The percentage of animals with tumors in this group at all times of observation was significantly lower than in the previous group, but virtually indistinguishable from them in the group of animals receiving DMBA alone.

In the second experiment 10 mg DMBA was given in two doses at an interval of 5 days. Under these experimental conditions DMBA was less toxic and only five of 20 rats died with

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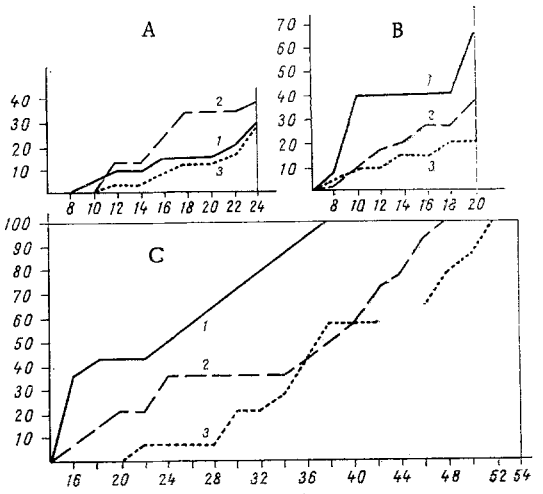

Fig. 1. Effect of CAP and DMC on induction of mammary gland tumors by DMBA. Abscissa, time after administration of DMBA (in weeks); ordinate, number of animals with tumors (in % of initial number in that group). CAP and DMC given in a dose of 1 mg/g 1.5-2 h before the carcinogen. A) Animals killed 24 weeks after a single dose of 10 mg DMBA; B) 10 mg DMBA given in two doses each of 5 mg, at interval of 5 days, and animals killed 20 weeks later; C) 10 mg DMBA given in two doses with an interval of 7 days and animals killed 64 weeks later. Original number of animals in group in A, B, and C was: DMBA 21, 20, and 14; DMBA + CAP 24, 29, and 14; DMBA + DMC 26, 20, and 14 respectively. In B and C decrease in rate of appearance of tumors after administration of DMBA with CAP or DMC relative to rate after administration of DMBA alone was statistically significant ($T > T_{05}$). 1) DMBA, 2) DMBA + CAP, 3) DMBA + DMC.

adrenal necrosis. These rats also were disregarded when the percentage of animals with tumors was calculated. The first tumor in this group appeared after 8 weeks (Fig. 1B) in one (6.6%) of 15 rats. The number of animals with tumors after 20 weeks was 66.7%

In the group of animals receiving CAP and DMBA the first tumor also appeared after 8 weeks in one (3.4%) of 29 rats. By the end of the experiments tumors had developed in only 37.9% of the rats compared with 66.7% receiving DMBA alone.

In rats receiving DMC and DMBA the first tumor also was found after 8 weeks in one (5%) of 20 rats. Tumors had developed after 20 weeks in only 20% of the animals.

CAP and DMC in these experiments, incidentally, completely prevented any toxic action of DMBA.

In the third experiment 10 mg DMBA also was given in two doses, but the interval between them was increased to 7 days. Under these experimental conditions none of the rats died from the toxic action of the carcinogen.

In rats receiving DMBA the first tumors were found after 16 weeks in five (35.7%) of the 14 animals (Fig. 1C). Throughout the subsequent period of observation an increase in the number of animals with tumors was noted, and after 38 weeks 100% of the rats had developed tumors.

In rats receiving CAP and DMBA the first tumor also appeared in the 16th week (in one of 14 rats, 7.1%). However, it was not until 48 weeks that tumors had developed in 100% of the animals, i.e., 10 weeks later than in animals receiving the carcinogen alone.

In animals receiving DMC and DMBA the latent period of onset of the tumors was increased to 22 weeks. At that time one (7.1%) of the 14 rats had a tumor, and not until 52 weeks after administration of the carcinogen were tumors found in 100% of the animals, i.e., 14 weeks later than in the animals receiving DMBA alone and 4 weeks later than in animals receiving CAP and DMBA.

The protective action of CAP and DMC in this experiment also was expressed as an increase in the length of survival of the rats: Animals receiving DMBA alone died 47 weeks after administration of the carcinogen, whereas when CAP and DMC were given, 29 and 63.3% of rats respectively were still alive even after 64 weeks.

Histological examination of the mammary gland tumors revealed carcinomas and fibroadenomas, with occasional adenofibromas and adenomas. In the early periods of observation (before the 24th week) the number of benign and malignant tumors in all groups was about the same, whereas later (after the 36th week) the number of malignant tumors increased considerably in all groups.

Hence, in the first experiment CAP and DMC did not prevent the appearance of mammary gland tumors. Indeed, CAP actually caused them to appear a little sooner. In the 2nd and 3rd experiments these compounds gave an inhibitory effect on carcinogenesis, but the strength of their protective action differed and was greater in the last experiment. These differences can be understood if the magnitude of the toxic action of DMBA is taken into account. In the first experiment, in the group of animals receiving DMBA alone, 40% of the rats died with necrosis in the adrenal cortex, whereas later the number of animals with tumors in this group increased very slowly. In the second experiments the toxicity of DMBA was only half as great: Only 20% of the rats died and tumors appeared much more rapidly. In the third experiments the carcinogen had no toxic action whatsoever and the number of animals with tumors increased faster still. Meanwhile, in animals receiving DMBA and CAP or DMC, completely preventing any toxic action of DMBA, the rate of appearance of tumors in the analogous group was practically the same in all three experiments. In this case the results of the first experiment can evidently be explained not by the absence of the protective effect of CAP and DMC, but by the greater toxicity of the DMBA toward the animals.

The protective action of CAP and DMC observed in the present experiments was reflected in a decrease in the percentage of animals with tumors at all times of observation, and in the case of DMC, by the later appearance of the first tumor. Although during prolonged observation on animals receiving DMBA with CAP or DMC tumors still developed in 100% of cases, this occurred 10-14 weeks later than in animals receiving DMBA alone.

These facts are evidence that the anticarcinogenic effect of CAP and DMC is linked with an increase in the latent period of tumor development rather than with a decrease in the total number of tumors which can be induced by DMBA. A similar mechanism of protection and the more marked effect of DMC were observed previously [1, 6].

The question of the mechanisms of the protective action of CAP and DMC has been widely discussed in the literature. The possible role of the effect of these compounds on the microsomal nonspecific oxidase system [10, 14] and also their action on binding of active metabolites with cell macromolecules [9, 13], a change in the RNA/DNA ratio, an increase in the half-life period of RNA, and an increase in the activity of soluble RNA-polymerase in the case of hepatic carcinogenesis [8, 11, 15] have been examined.

So far as DMBA is concerned, experiments on cultures of mouse cells have shown that CAP and DMC do not affect the intensity of metabolism of this carcinogen; the antitoxic effect, moreover, correlated with disturbance of binding of the carcinogen with DNA and RNA [4].

The anticarcinogenic effect of CAP and DMC in relation to DMBA may perhaps have a similar mechanism. However, this hypothesis requires further experimental verification.

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MUTAGENIC AND CARCINOGENIC ACTION OF SOME POLYCYCLIC AROMATIC HYDROCARBONS

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The carcinogenic (on mice) and mutagenic (on bacteria *Salmonella typhimurium* TA-98 and TA-100) action of benz(a)pyrene (BP) and its derivatives: 6-methyl-, 6-formyl-, 6-chloro-, 6-hydroxy-, 6-acetoxy-, 6-methoxy-, and 4(5)-methoxy-BP was investigated. Powerful carcinogens (BP, 6-methyl- and 6-formyl-BP) were shown to cause mutations in both strains of bacteria. Weakly carcinogenic compounds [6-chloro-, 6-methoxy-, and 4(5)methoxy-BP] and noncarcinogenic (6-acetoxy- and 6-hydroxy-BP) compounds either were nonmutagenic or induced mutations in bacteria of only one of the two strains. Differences between the carcinogenic and mutagenic actions of the various compounds were not related to the velocity of their oxidation in an enzymic system.

KEY WORDS: Carcinogenesis; mutagenesis; polycyclic hydrocarbons

Compounds foreign to the body, including polycyclic aromatic hydrocarbons (PAH), are oxidized by enzymes of the endoplasmic reticulum of the cell with the formation of highly active compounds, which are responsible for the biological effect of the PAH. As a result of the high chemical activity of these metabolites and, consequently, of their instability *in vivo*, it is virtually impossible to isolate them. However, by using an appropriate acceptor system for active metabolites, in the presence of enzymes oxidizing PAH, it is possible to determine whether a particular compound, as a result of enzymic conversion, can form highly active agents. Various mutants of bacteria can be used as acceptors of active metabolites of PAH.

In this investigation the carcinogenic and mutagenic actions of benz(a)pyrene (BP) derivatives similar in structure but with different carcinogenic activity were compared. The following compounds were tested: BP, 6-methyl-BP, 6-formyl-BP, 6-chloro-BP, 6-hydroxy-BP, 6-acetoxy-BP, 6-methoxy-BP, and 4(5)-methoxy-BP. Bacteria of strains *Salmonella typhimurium* TA-98 and TA-100 were used as acceptors of active metabolites of PAH [8].

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