Prevention by Spironolactone of 7,12-Dimethylbenz(*a*)anthracene-induced Adrenal Necrosis* (34103)

KALMAN KOVACS AND ARPAD SOMOGYI
(Introduced by Hans Selye)
Institute of Experimental Medicine and Surgery, University of Montreal, Montreal, Canada

Huggins and Morii (1) reported in 1961 that 7,12-dimethylbenz(*a*)anthracene (DMBA) given to adult rats induces massive hemorrhagic necrosis in the inner layers of the adrenal cortex. In the following years these results were confirmed and extended by several authors (2–6). Further experiments showed that various procedures (administration of methopyrapone, certain azo dyes, and polynuclear aromatic hydrocarbons; *e.g.*, pretreatment with methylcholanthrene or DMBA itself, or hypophysectomy) prevent adrenal injury in DMBA-treated rats (1, 3, 7–11). However, all these protective measures possess toxic effects and/or cause marked changes in hormonal equilibrium. We have now found that DMBA-induced adrenal necrosis is prevented by spironolactone, a hormonally inactive nontoxic steroid lactone. This drug is widely used in clinical medicine as an aldosterone antagonist. It inhibits the effect of aldosterone in the renal tubules and does not directly interfere with the formation of corticoids.

Materials and Methods. Female Sprague-Dawley rats (Holtzman Farms, Madison, Wisconsin) weighing 180–210 g and kept *ad libitum* on Purina Laboratory Chow (Ralston Purina Co. of Canada) and tap water, were divided into two equal groups of ten animals each. A single dose of 40 mg DMBA (Eastman Organic Chemicals, Rochester, New York) dissolved in 2 ml of corn oil was given by stomach tube to both groups. In addition, one of the groups received spironolactone (Aldactone, G. D. Searle & Co., Chicago, Illinois) at a dose of 10 mg/100 g body weight in 1 ml of distilled water by mouth twice daily for 6 consecutive days beginning 2 days prior to the DMBA gavage. This experiment was performed three times.

The survivors were killed with chloroform on Day 4 after DMBA administration; the adrenals were excised, fixed in alcohol–formol and embedded in paraffin. Sections were stained with hematoxylin–phloxine.

Results. The results of these three experiments are summarized in Table I. Hemorrhagic necrosis of the adrenal glands was found in 25 of the 30 DMBA-treated rats, but failed to develop in any of the animals treated additionally with spironolactone (Fig. 1). In additional experiments, not reported here, we observed a similar inhibitory effect when DMBA was given intravenously under otherwise identical conditions. Hence, the possibility of a local chemical interaction between DMBA and spironolactone in the gastrointestinal tract can be excluded.

TABLE I. Effect of Spironolactone on Mortality and Adrenal Necrosis Induced by DMBA.

Treatment	No. of rats	Mortality	Incidence of adrenal necrosis
DMBA	30	3	25
DMBA + spironolactone	30	0	0

Discussion. It is generally accepted that spironolactone acts exclusively on the renal tubules by antagonizing the effect of aldosterone, thus increasing sodium excretion and causing potassium retention. Our results re-

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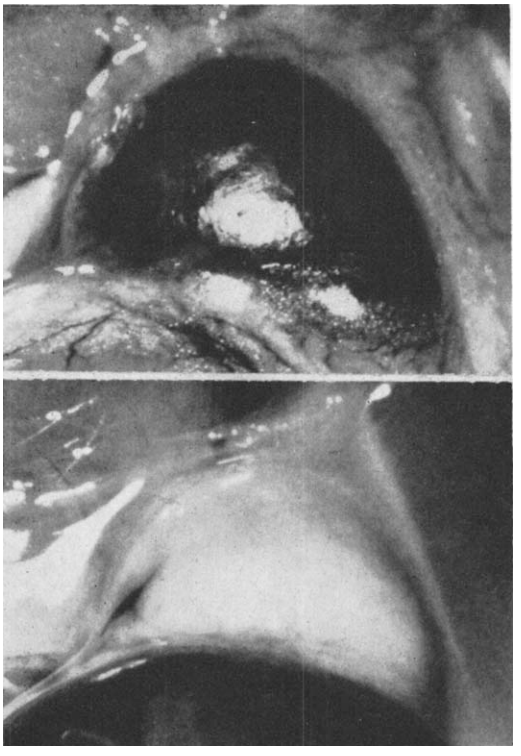

FIG. 1. Prevention of DMBA-induced hemorrhagic adrenal necrosis by spironolactone. Upper: Hemorrhagic necrosis of the adrenal of the rat 4 days after the oral administraition of DMBA. (Note the pronounced periadrenal edema, causing light reflections at the cranial pole of the adjacent kidney.) Lower: No lesions are seen in the adrenal of the rat receiving spironolactone additionally.

veal a hitherto unknown extrarenal action of this drug.

The mechanism whereby spironolactone prevents DMBA-induced adrenal necrosis is not clear. Currie *et al.* (7), while observing adrenal protection from methopyrapone (Metopirone), an adrenal 11β-hydroxylase inhibitor, supposed that adrenocortical necrosis depends upon active corticosteroid synthesis in the adrenal cortex at the time of exposure to the polycyclic hydrocarbon. However, this hypothesis does not offer an explanation for the preventive action of spironolactone, since this compound does not directly interfere with the formation of corticoids. Recently it was assumed that methopyrapone and several other protective agents do not act at the adrenal level but stimulate drug-metabolizing enzymes in the liver, thus transforming DMBA into inactive metabolites (11, 12). In fact, it has been demonstrated that various microsomal enzymes are induced in the liver of rats treated with a number of agents which prevent the adrenocorticolytic effect of DMBA (9, 13, 14). Furthermore, prior treatment with ethionine, an inhibitor of protein synthesis, abolishes the protective effect of these compounds (9, 11).

On the basis of this information it seems reasonable to suppose that a similar mechanism is involved in the preventive action of spironolactone. This view is supported by the previous findings of Selye and his associates who have demonstrated that spironolactone inhibits steroid and phenobarbital anesthesia (15) and the toxic effects of digitalis glucosides (16). It is known that these reactions are influenced by intrahepatic microsomal enzymes (17, 18). Furthermore, the toxicity resulting from the combined treatment with dihydrotachysterol and Na_2HPO_4 was shown to be counteracted by the administration of SC11927, an antialdosterone spironolactone derivative (19).

Experiments are under way to elucidate the mechanism of this protective action and to investigate the possible prevention with spironolactone of DMBA-induced mammary tumors as it has been shown that the inhibition of these tumors runs parallel with adrenal protection (20).

Summary. Administration of spironolactone protects the rat against 7,12-dimethylbenz (*a*)anthracene-induced adrenal necrosis.

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