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Antiprostatic Effects of a Nitrogen Mustard of Estriol[1] (38657)

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The development of chemotherapeutic agents consisting of antimetabolites and steroid hormones, e.g., estrogens, is based on the known intracellular localization and action of certain steroids (1) and the possibility that the estrogens could serve as carriers for the antimetabolites into the desired cells. These considerations apply in particular to cancer of the prostate and its relation to estradiol-17β (E_2) and other estrogenic compounds (e.g., stilbestrol). It is known that E_2 localizes in the prostatic cells (2–6) and that this steroid hormone leads to remarkable atrophy of the gland (7) through effects on the hypothalamic–pituitary system, which results in greatly decreased testosterone synthesis by the testes, and possibly through a direct effect of the estrogens on the prostatic cells (8–10). Thus, if preferential localization of E_2 in prostatic cells can still take place when an antimetabolite is conjugated with E_2, the intracellular hydrolysis of the complex would then afford the antimetabolite a direct action intracellularly and also lead to estrogenic effects.

The synthesis of a chemical ester of a nitrogen mustard with E_2 (Estracyt) has led to its use in cancer of the prostate with good palliative effects (11). In addition, the antiprostatic effects of Estracyt have been studied in dogs and rats (12).

Since in some clinical circumstances the metabolic effects of E_2 may not be beneficial, the synthesis of a conjugate of a nitrogen mustard with estriol (E_3) was accomplished. The latter steroid is known to have much less evident estrogenic effects, when compared to those of E_2, and has been shown to localize in the prostate to an extent similar to that of E_2 (4, 6). The present study was an attempt to examine the antiprostatic effects of such an estriol-mustard (E_3-mustard) in dogs and rats. The approaches used in the present study were very similar to those employed by us previously in studying Estracyt (12).

Materials and Methods. For the prostatic deposition of labeled E_3 and testosterone (T) 6 mongrel adult male dogs (weight 10–15 kg) were used. Mixtures of differently labeled steroids (50 μCi of ^{3}H–E_3 and 10 μCi of ^{14}C-T) were injected intravenously as previously described (4, 6, 16). Biopsies of different tissues were obtained at varying time intervals following the iv injection of the labeled steroids and the radioactivity in the tissues determined by previously described methods. An estriol mustard [estriol-3-bis(2-chloroethyl)carbamate-17-dihydrogen phosphate] was synthesized at the AB Leo Co. in Helsingborg, Sweden and kindly supplied to us by Drs. B. Högberg and J. Müntzing (Fig. 1). The E_3-mustard was injected iv twice daily (2.5 mg/kg) for 2 days and again 2–3 hr. prior to the injection of the labeled steroids on the third day. The effect of such treatment on the deposition of the labeled T and E_3 were compared with results in untreated dogs.

E_3-6,7-^{3}H (50 mCi/μM), E_2-4-^{14}C (50 μCi/μM), T-1,2-^{3}H (50 mCi/μM) and T-4-^{14}C (50 μCi/μM) were purchased from the New England Nuclear Corp., Boston, MA and checked for purity by paper chromatography.

For the measurement of prostatic 5α-reductase and arginase activities the methods of Shimazaki *et al.* (13) and Yamanaka *et al.* (14) were used, respectively, with minor modifications (12).

Studies on the deposition of radioactivity of injected labeled T and E_2 in normal and castrated Wistar rats followed procedures described previously (12).

Results. In Figs. 2 and 3 are shown the changes with time in the prostatic uptake of radioactivity associated with the injected labeled steroids in untreated and treated dogs. A definite inhibition of prostatic uptake of

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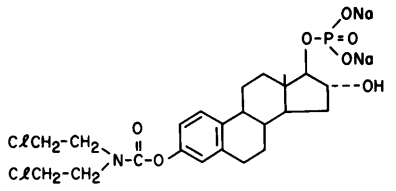

Estriol-3-N-bis(2-chloroethyl) carbamate--17-phosphate, disodium salt

FIG. 1. Chemical formula of E_3-mustard showing it to be an ester of a nitrogen mustard and estriol (E_3), the former being attached to the latter at position 3. A phosphate group is present at position 17. The E_3 mustard was manufactured by AB Leo Co. and consists of estriol-3-bis (2-chloroethyl) carbamate-17-dihydrogen phosphate, disodium salt.

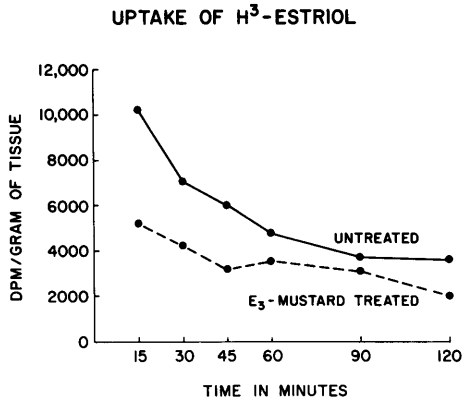

FIG. 2. Mean concentrations of radioactivity associated with injected labeled E_3 in the prostates of two dogs treated with E_3-mustard. The latter was injected intravenously twice daily (2.5 mg/kg) for 2 days and again 2–3 hr prior to the injection of the labeled steroid (E_3) on the third day. The mean concentrations of radioactivity in the prostates of four untreated dogs are shown for comparison. The lower levels of radioactivity following the E_3-mustard treatment are statistically significant ($P < 0.05$).

labeled E_3 in the dogs pretreated with the E_3-mustard, as compared with the results of the untreated dogs, is evident. In contrast, the uptake of the labeled T was significantly increased above that observed in untreated dogs.

In Tables I and II are shown the mean values of radioactivity concentrations in various tissues of untreated and treated dogs. There was a statistically significant decrease in the concentration of E_3 radioactivity in all tissues examined in the treated animals as compared to the untreated ones ($P < 0.05$) (Table I). On the other hand, there was a significant increase in the radioactivity concentrations of labeled T only in the prostate ($P < 0.05$), a significant decrease in the blood and no significant changes in other tissues ($P > 0.5$) (Table II).

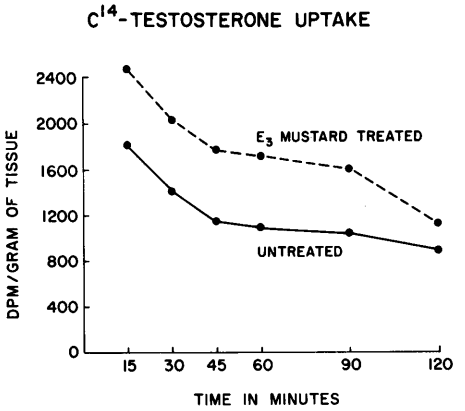

FIG. 3. Mean concentrations of radioactivity associated with injected labeled testosterone (T) in the prostates of two dogs treated with E_3-mustard. The latter was injected intravenously twice daily (2.5 mg/kg) for two days and again 2–3 hr prior to the injection of the labeled steroid (T) on the third day. It should be pointed out that the labeled T was injected as an admixture with the labeled E_3. The mean concentration of radioactivity in the prostates of four untreated dogs are shown for comparison. The increased levels of radioactivity following the E_3-mustard therapy are statistically significant ($P < 0.05$).

When the estriol mustard was administered to rats for 2 days (5 mg/day) there was a definite effect on the weights of the ventral and dorsolateral prostates. Thus, the mean ventral and dorsolateral prostatic weights in 10 control rats were 426.5 mg $\pm$3.2 (SEM) and 254.5 mg $\pm$ 8 (SEM), respectively. After the administration of the estriol mustard the mean value for the ventral prostate in 20 rats was 301.5 mg $\pm$ 11.1 and for the dorsolateral prostate 196.0 mg $\pm$ 5.9. No significant changes in body weight occurred during this 2-day period of administration. Thus, E_3-mustard produced a very significant decrease in rat prostatic weights, even when given for a short period of 2 days.

The results of labeled ^{3}H-T and ^{14}C-E_2 deposition in rat tissues are shown in Table III. When the results of group I (control in-

TABLE I. RADIOACTIVITY CONCENTRATIONS IN CANINE TISSUES AFTER THE INTRAVENOUS INJECTIONS OF 50 μCi OF ^{3}H-E_3 IN UNTREATED DOGS AND THOSE TREATED WITH E_3-MUSTARD[a]

Group	Muscle	Testes	Blood	Prostate	Adrenals	Pancreas	Kidney	Liver
Untreated (4 dogs)	2338	1967	3607	6474	6122	30,016	11,121	30,535
	±230	±200	±443	±869	±1654	±2093	±2093	±9564
Treated with Estriol mustard[b] (2 dogs)	927	656	1458	3464	1948	16,947	4319	12,779
	±103	±101	±182	±459	±517	±2835	±640	±308

[a] Mean values ± SEM of disintegrations per minute per gram or milliliter of all specimens in each group.

[b] $P < 0.05$ in all organs between untreated and treated dogs.

In this and in the following table, the radioactive steroids were dissolved in 20 ml of saline and injected *iv* over a period of 1 min and the blood and tissue samples were obtained at 15, 30, 45, 60, 90, and 120 mins after the injection.

TABLE II. RADIOACTIVITY CONCENTRATIONS IN CANINE TISSUES AFTER INTRAVENOUS INJECTION OF 10 μCi OF ^{14}C-TESTOSTERONE IN UNTREATED DOGS AND THOSE TREATED WITH E_3-MUSTARD.[a]

Group	Muscle	Testes	Blood[b]	Prostate[b]	Adrenals	Pancreas	Kidney	Liver
Untreated (4 dogs)	410	546	706	1319	943	852	1826	7416
	±39	±84	±98	±121	±172	±148	±308	±2170
Treated with Estriol mustard (2 dogs)	391	479	529	1757	877	1016	1398	5989
	±44	±77	±77	±134	±200	±179	±178	±1358

[a] Mean values ± SEM of disintegrations per minute per gram or milliliter of all specimens in each group.

[b] $P < 0.05$ between treated and untreated dogs; the values in all other tissues were not significantly different.

tact) are compared with those of group III (intact pretreated with E_3-mustard) it is evident that there was a decreased deposition of the labeled T in the ventral prostate, levator ani muscle and possibly in the pancreas, with an increased radioactivity concentration being present in the dorsolateral prostate. In the case of labeled E_2, there was a decreased deposition in the ventral prostate, seminal vesicles and levator ani. The results in untreated castrated rats (group II) and those of treated castrated rats (group IV) were not remarkably divergent, except for a high concentration of labeled T in the seminal vesicles and of E_3 in the muscle in the latter group.

The effects of the E_3-mustard on rat prostatic 5α-reductase and arginase activities were determined. These enzymes are essential in maintaining prostatic integrity and function (3, 12). Because of the limited supply of the mustard, the effects of only one dose level (1 mg/kg) were ascertained. The drug was given intraperitoneally daily for 4 days to two groups of seven rats each, one group serving as a control and the other as the experimental one. Using the control value as 100% (μmoles of urea/min/g) the arginase activity declined to a mean of 50 ± 7% (SEM) of the control value in the ventral prostate and no change was observed in activity of the dorsolateral gland. The 5α-reductase activity (conversion of T to DHT) declined to a mean of 34 ± 6% of the control in the ventral gland and 71 ± 4% in the dorsolateral prostate. These results are very similar to those obtained previously with the mustard of estradiol-17β (Estracyt) (12). In both cases definite effects on the 5α-reductase and arginase activities were obtained in the ventral prostate of the rat, with lesser decreases being observed in the 5α-reductase activity in the dorsolateral gland.

Discussion. The data of the present study indicate that the E_3-mustard employed elicited prostatic effects very similar to those observed following the administration of an E_2-mustard (Estracyt) (12). This was shown

TABLE III. EFFECTS OF E_3-MUSTARD ON THE *In Vivo* DEPOSITION OF INJECTED ^{3}H-T (20 μCi) IN INTACT AND CASTRATED MALE RATS.

Tissue	Group I[d]	Group II[e]	Group III[f]	Group IV[g]
Ventral prostate	91.3 ± 56.7 (1.8)[b]	58.5 ± 68.7 (1.4)	69.6 ± 48.2 (1.8)	88.8 ± 13.7 (1.8)
	2.8 ± 1.4 (2.2)[b]	0.8 ± 0.9 (0.8)	0.8 ± 0.5 (0.8)	1.2 ± .07 (0.4)
	44.0 ± 3.1[c]	86.5 ± 14.9	78.9 ± 9.2	71.8 ± 8.3
Dorsolateral prostate	86.5 ± 43.0 (2.0)[b]	157.7 ± 189.6 (3.6)	155.2 ± 110.8 (3.9)	154.3 ± 24.4 (2.1)
	1.7 ± 0.6 (1.9)[b]	2.4 ± 2.7 (3.1)	1.6 ± 0.8 (1.9)	1.8 ± 0.3 (0.3)
	48.9 ± 5.6[c]	58.2 ± 14.7	81.8 ± 26.0	86.8 ± 14.2
Seminal vesicles	70.2 ± 42.4 (1.5)[b]	56.4 ± 62.9 (1.4)	77.6 ± 62.6 .1.9)	119.9 ± 29.1 (2.4)
	1.3 ± 0.7 (1.3)[b]	0.9 ± 0.8 (1.7)	0.6 ± 0.5 (0.5)	1.2 ± 0.2 (0.3)
	50.3 ± 4.1[c]	51.4 ± 26.6	126.3 ± 28.9	110.5 ± 11.8
Levator ani	44.0 ± 37.7 (0.8)[b]	30.1 ± 33.0 (0.8)	23.0 ± 12.4 (0.7)	42.4 ± 8.6 (0.8)
	0.9 ± 0.8 (0.8)[b]	0.5 ± 0.7 (0.6)	0.5 ± 0.4 .0.4)	0.9 ± 0.2 (0.2)
	44.9 ± 3.9[c]	75.6 ± 32.4	67.4 ± 22.4	45.6 ± 1.1
Testes	55.3 ± 26.2 (1.2)[a]		41.9 ± 21.4 (1.2)	
	1.2 ± 0.6 (1.2)[b]		0.8 ± 0.4 .0.9)	
	46.8 ± 1.2[c]		56.1 ± 5.6	
Pancreas	125.4 ± 45.1 (3.2)[a]	93.7 ± 85.4 (2.9)	84.9 ± 33.4 (2.7)	109.0 ± 9.0 (2.2)
	5.8 ± 2.7 (6.0)[a]	11.8 ± 15.6 (10.4)	7.0 ± 3.8 (7.8)	9.4 ± 2.0 (2.0)
	27.7 ± 11.3[c]	23.7 ± 24.0	17.8 ± 8.1	12.7 ± 4.1
Kidney	144.4 ± 49.4 (3.7)[a]	156.0 ± 101.5 (6.0)	114.2 ± 31.6 (4.0)	148.1 ± 24.1 (3.0)
	3.6 ± 1.1 (4.2)[b]	3.6 ± 2.4 (8.3)	2.6 ± 0.8 (4.2)	3.2 ± 0.3 (0.7)
	40.0 ± 2.2[c]	43.8 ± 0.4	43.8 ± 2.5	46.7 ± 3.8
Muscle	43.1 ± 20.0[a]	34.3 ± 33.9	32.8 ± 15.4	49.6 ± 2.0
	1.0 ± 0.5[b]	0.8 ± 0.6	1.2 ± 0.9	6.7 ± 0.3
	45.2 ± 2.2[c]	54.6 ± 22.0	40.3 ± 11.7	10.2 ± 5.6

[a] All numbers on these lines refer to tritium. Results are shown as dpm × 10^{-3}.
[b] All numbers on these lines refer to carbon-14. Results are shown as dpm × 10^{-3}.
[c] The numbers in parentheses are ratios of dpm in a gram of tissue to those in a gram of muscle.
[d] Group I—Control Intact.
[e] Group II—Control-Castrated.
[f] Group III—Intact Pretreated-with E_3-mustard.
[g] Group IV—Castrated Pretreated with E_3-mustard.
All numbers are dpm/g of tissue ± standard error of the mean (SEM).
The labeled steroids were dissolved in 1 ml of saline and injected iv and the tissue samples obtained 15 min after the in ection.

by the reduced deposition in the canine prostate of labeled E_3, decreased weight of the ventral and dorsolateral prostates of the rat after only 2 days' treatment with E_3-mustard and decreased deposition of T in the ventral prostate of the rats. Even though some of the results can be seen following the administration of E_3 or E_2 alone (4, 6, 12), several of the effects, e.g., decreased deposition of T in some of the organs (ventral prostate, levator ani) known to have androgen receptors, have not been seen with estrogens alone. This effect may be due to the action of the E_3-mustard or to the liberated mustard moiety acting alone or the two compounds acting in concert.

The increased concentration of radioactivity in the dog prostate observed following the administration of the E_3-mustard, as well as the increase of deposition of labeled DHT following Estracyt treatment previously reported by us (12), deserve some comment. No increased deposition in the dog prostate of labeled T or DHT was observed following E_3 (5 mg/day) or E_2 administration. The increased deposition of T and DHT concentrations following E_3-mustard or Estracyt administration, respectively, may be due to (a) either an effect on the hypothalamic–pituitary–gonadal axis resulting from much higher amounts (about 25 mg/day) of estrogen released in the body from the conjugated mustard, leading to decreased testosterone synthesis, or to (b) a direct effect on testicular function, either by the estrogen and/or mustard, and consequent reduced testosterone synthesis. In either case, in short term experiments of only 2 days, the decreased circulating amount of testosterone would ultimately result in decrease stores of testosterone and DHT in the prostatic cells and afford more binding sites for the labeled T or DHT.

Some of the considerations regarding the

mechanism of action of an estrogen-mustard and its fate in the body have been discussed by us previously (12). These considerations relate to the extent of hydrolysis of the conjugate mustard before it reaches the prostate (or any other target organ), the ability of the target tissue (e.g., prostate) to split the estrogen from the mustard appropriately and the capacity to dephosphorylate the estrogen at the 17-position for its full activity. Since we have shown that the metabolism of estrogen–mustards and their conjugates differ among animals (including the human), the effects of the E_3-mustard will be the result of the free estrogen and/or mustard. The findings in the present study point to the antiprostatic effects as being a result of the combined action of E_3 and/or the nitrogen mustard, either acting singly or in concert.

Summary. The chemical ester of a nitrogen mustard with estriol was tested for its antiprostatic effects in dogs and rats. The E_3-mustard was shown to interfere with the uptake of labeled estriol in the dog prostate and by the ventral prostate of the rat; and to increase the uptake of the radioactivity associated with testosterone in the dog prostate. The weights of the ventral and dorsolateral prostates of the rat were significantly reduced following the administration of E_3-mustard for 2 days. The results are interpreted to be very similar to those obtained with the mustard of E_2 (Estracyt) and the effects are probably a combination of the actions of the released estrogen (E_3) and/or mustard, either adting individually or in concert.

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