Studies on the Antiprostatic Action of Estracyt, a Nitrogen Mustard of Estradiol

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SUMMARY

Estracyt, a chemical ester of a nitrogen mustard with estradiol-17β, was tested for its antiprostatic effects in dogs and rats. Estracyt was shown to interfere with the uptake of labeled estriol by the dog prostate, without affecting that of testosterone and dihydrotestosterone. Similarly, it affected the uptake of estradiol-17β by the prostates of castrated rats, although in this animal it also interfered with the uptake of dihydrotestosterone. Estracyt greatly reduced the prostatic secretion in dogs. The results of these short-term experiments indicate that Estracyt affects a number of different parameters in the prostates of dogs and rats. Possible mechanisms of action of Estracyt were discussed.

INTRODUCTION

The concept behind the development of chemotherapeutic agents consisting of chemical conjugates, usually esters, of steroid hormones and antimetabolites is based on relatively recent findings concerning the possible mechanism of action of these hormones, particularly the estrogens, at the cellular level. Thus, it has been demonstrated that special receptor proteins for E₂ and other estrogens are present in the target organs of these hormones, e.g., uterus, breast (4). Similar receptor proteins for androgens (testosterone, DHT) are present in male target organs, e.g., prostate, seminal vesicles (25). Furthermore, in the prostate of several species, the presence of intracellular receptor proteins for both estrogens and androgens has been demonstrated recently (2, 3, 7).

It has been inferred from the above that the interaction of the cytoplasmic receptor protein with a steroid leads to modification of this protein, which then associates with the nucleus, thus influencing transcription of the DNA of the nuclear chromatin. This process is essential for the control of cellular metabolism.

It is also inferred that the presence of receptor proteins in a cell may indicate that the steroids with high affinity for these proteins play an important role in the integrity and metabolism of such cells. Hence, it would appear that both androgens and estrogens may play a role in prostatic cells and possibly in those of other organs.

Estrogens have been shown to affect the prostate in a number of animals, including the human (14). The synthesis of a nitrogen mustard of E₂ was thought to be of promise in the treatment of cancer of the prostate, if the E₂ moiety of the molecule could be used as a carrier ("piggy back") for the antimetabolite into the cells and if important groups necessary for E₂ to associate with receptor proteins in the prostatic cells were not affected by the nitrogen mustard moiety. Recently, a nitrogen mustard of E₂, Estracyt (Chart 1), has been shown to be effective, at least palliatively, in cancer of the prostate in a number of clinical studies in Europe (1, 5, 6, 12, 13) and at our Institute (11). The purpose of the present study was to ascertain the possible mechanism of action and the prostatic effects of Estracyt in dogs and rats, in order to understand more of the effects of the drug in human subjects.

The plan of study was very similar to that we have utilized in ascertaining the effects of other antiprostatic agents (20–22) and included the following.

1. The effects of Estracyt on the prostatic uptake of labeled testosterone, DHT, E₃, and E₂ in the dog and rat. These steroids have been shown to localize in the prostates of these animals (3, 7, 20, 21) and, hence, it was thought worthwhile to establish the effects of Estracyt on the prostatic uptake of these steroids.

2. Effect on prostatic fluid excretion and composition in the dog.

3. Effect on rat and dog prostatic 5α-reductase and arginase activities. The 5α-reductase is essential in the reduction of testosterone to DHT, and DHT being necessary for prostatic integrity and function. Although the exact role of arginase is uncertain, it and 5α-reductase are profoundly under the influence of androgens and possibly estrogens (18, 26).

MATERIALS AND METHODS

For tissue deposition studies, male mongrel dogs weighing 10 to 15 kg (2 to 4 years old) were used. Mixtures of differently labeled steroids (50 μCi DHT-3H and 10 μCi E₂-14C or 50 μCi E₃-3H and 10 μCi testosterone 14C) were injected as previously described (9, 20, 21). Biopsies of various tissues were taken at 15, 30, 45, 60, 90, and 120 min following the i.v. injection of the steroids. Details of the preparation of tissue samples for the determination of...
radioactivity and the methods utilized with the tissue oxidizer have been previously described (8, 9, 21).

The effects of Estracyt on the deposition of labeled testosterone, DHT, and E2 in the prostate and other tissues of the dog were examined following the i.v. administration of a commercial preparation of the drug (A. B. Leo, Helsingborg, Sweden; Batch X4645 of Estracyt), which was given twice daily (2.5 mg/kg) for 2 days and then 2 to 3 hr prior to the injection of the labeled steroids on the 3rd day. Since Estracyt is phosphorylated at position 17 of the estradiol, the effects of E2-phosphate on the deposition of labeled steroids (DHT and E2) were determined in dogs. The E2-phosphate was given to 3 dogs (1.7 mg/kg/day i.v. twice daily in 0.9% NaCl solution) for 2.5 days, and then 50 µCi of DHT-3H and 10 µCi of E2-14C were injected i.v. 2 to 3 hr after the last dose of the estrogen phosphate. The results were compared with those of untreated normal male dogs of similar age and weight.

DHT-1,2-3H (48 mCi/µM), E2-4-14C (50 µCi/µM), E2-4-13C (37 µCi/µM), E3-6,7-3H (50 mCi/µM), and testosterone 4-14C (50 µCi/µM) were purchased from New England Nuclear, Boston, Mass., and checked for purity by paper chromatography. The A. B. Leo Co. kindly supplied us with nonlabeled Estracyt, dephosphorylated Estracyt, and E2-phosphate.

The influence of Estracyt on the deposition of labeled testosterone and E2 in intact and castrated rats was determined in 2 separate experiments. In 1 set of experiments Estracyt was given p.o. by gavage for 2 days (25 mg/kg) and 30 min before the i.v. injection of the labeled steroids on the 3rd day to intact and castrated rats. The animals were anesthetized 15 min after the injection of the labeled steroids, and the various tissues and organs were excised. The Estracyt was given to the castrated rat 2 days after the surgery. In another set of experiments the Estracyt was given to intact and castrated rats for 4 days, starting on the day of castration.

Prostatic fluid from dogs was obtained through a cyster-preputiostomy-type fistula after i.v. injection with pilocarpine (0.7 mg/kg) (10). Fluid collections were made every 10 min after injection of the labeled steroid mixture. We found that radioactivity in these samples is best determined by the use of the sample oxidizer; otherwise, variable results were obtained when direct determinations of radioactivity were made.

The effects of Estracyt on prostatic secretion in dogs were determined after the animals were treated with the drug for 7 days (2.5 mg/kg i.v. twice daily). In addition to various chemical determinations on the fluids, the excretion of radioactivity following the injection of labeled DHT-3H and E2-14C was determined.

Measurement of prostatic 5α-reductase activity was made using a modification of the method of Shimazaki et al. (18). A 50% homogenate of prostatic tissue was made by grinding the specimen by hand in an all-glass Potter-Elvehjem homogenizer to which had been added a volume equal to the weight of the tissue of 50 mM Tris-HCl buffer, pH 7.4. The homogenate was filtered through gauze, and the gauze was squeezed to drain out as much liquid as possible. Homogenate equivalent to 300 mg of wet weight of tissue was taken for 5α-reductase activity. Incubation with testosterone-14C for 1 hr at 37° was performed following the procedure of Shimazaki et al. (18). After addition of alcohol to the incubation medium, standard testosterone, DHT, and androstenedione were added; the mixture was extracted according to the method of Shimazaki et al. (18); and an initial paper chromatography was performed on the extract in 80% methanol in water and benzene. Two peaks separated with typical Rf values of 0.41 (testosterone) and a minor peak with an Rf of 0.63 (DHT and androstenedione). The 2nd peak was then eluted, acetylated, and rechromatographed in the system 80% methanol in water and heptane. This system separated androstenedione (Rf 0.39) from acetylated DHT (Rf 0.94). The DHT acetate and androstenedione were eluted and together with the testosterone peak from the Bush-3 chromatogram were quantitated for recovery by determining the UV absorption for androstenedione and testosterone. Arginase activity was determined by the method of Yamanaka et al. (26).

RESULTS

The Effects of Estracyt on the Prostatic Uptake of Labeled DHT, Testosterone, and E2 in the Dog. The changes with time in the prostatic uptake of radioactivity associated with injected labeled steroids in untreated and Estracyt treated dogs are shown in Charts 2 to 4. The steroids were injected i.v. in pairs (DHT-3H plus E2-14C and E2-3H plus testosterone-14C), but for graphic presentation the results for each steroid are shown individually. The results indicate a definite inhibition of uptake of the labeled E2 (Charts 1 and 3) in the prostate of the dogs following the administration of Estracyt for only 2 days. On the other hand, the uptake of the labeled DHT (Chart 2) was significantly increased, whereas that of labeled testosterone was not affected by the Estracyt treatment.

The levels of radioactivity shown in Charts 2 to 4 had a standard error of the mean that did not exceed 10% of the counts shown for any point in the figures and usually was in the range of ±5% of the dpm.

Statistical analysis of the differences between the values shown for control and Estracyt-treated dogs in Charts 2 to 4 revealed p values of <0.001 for all points in Charts 2 and 4.

Chart 1. Chemical formula of Estracyt, showing it to be an ester of a nitrogen mustard and estradiol-17β, the former being attached to the latter at position 3. A phosphate group is present at position 17. Estramustine phosphate is manufactured by the A. E. Leo Co. and consists of estradiol-3-bis(2-chloroethyl)carbamate-17-dihydrogen phosphate.

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Charts 2 and 3. Mean radioactivity levels in the canine prostate following treatment with Estracyt (2.5 mg/kg/day i.v. for 2.5 days to 3 dogs). The pair of steroids, estradiol-13C and DHT-3H was injected i.v., 10 μCi and 50 μCi, respectively. The mean levels of untreated dogs are shown for comparison. Estracyt caused a markedly decreased uptake of the E2-13C by the prostate. There was no decrease in the uptake of DHT-3H and, if anything, a somewhat increased concentration of radioactivity in the prostate. The values for the two separate groups of 3 untreated dogs are shown for comparison in Chart 3.

and p values of <0.005 in Chart 3 and indicate a very significant effect of Estracyt on the localization of the radioactivity of administered E2 and DHT in the prostate.

Since the E2 in Estracyt is phosphorylated at position 17, we next examined the effect of E2-phosphate on the deposition of DHT-3H (50 μCi) and E2-13C (10 μCi). The effects on the uptake of the labeled E2 by the prostate were similar to those of Estracyt or E2. No significant effects on the uptake of the labeled E2 and DHT were seen in other tissues, however, including the pancreas. The latter organ has been shown to concentrate significant amounts of E2 and E2-13C. This contrasts with data obtained following the administration of E2 or E2-13C to dogs (20), which does lead to a decreased deposition of labeled E2 or E2-13C in the pancreas, apparently the unlabeled steroids competing with the labeled ones for intracellular sites (16).

The Effects of Estracyt on the Uptake of Labeled Testosterone and E2 by Rat Tissues, Including the Prostate. When Estracyt was administered p.o. to male adult intact or castrated rats for 2 days (Table 1), a markedly decreased deposition of radioactivity following the i.v. injection of the pair of steroids (testosterone-3H and E2-13C) was observed as follows: E2 in the prostate of castrated rats, in the seminal vesicles of both intact and castrated animals, and in the pancreas of intact rats; testosterone in the prostate of castrated rats, and the levator ani, testes, and pancreas of intact rats. In all organs and tissues, except the pancreas, of the rats, both intact and castrated, the 3H/13C far exceeded the injected one (5/1) and indicates more rapid excretion of the labeled E2 than of testosterone. In the pancreas the ratio was actually decreased and is in keeping with the avid uptake of E2 by pancreatic tissue previously described by us (9). Parenthetically, the 3H/13C in the pancreas was not affected by Estracyt.

Effects of Estracyt on Prostatic Fluid Excretion and Composition in the Dog. Following 1 week of treatment of 4 dogs with Estracyt (2.5 mg/kg i.v. twice daily), the secretion of prostatic fluid totally stopped in 2 animals, and in the other 2 animals the volume decreased to 43 and 35% of the controls, respectively. The chemical composition of the fluid before and after the administration of Estracyt and the radioactivity excreted following the injection of the pair of steroids DHT-3H (50 μCi) and E2-13C (10 μCi) are shown in Table 2.

Estracyt definitely decreased the concentration of acid phosphatase (p < 0.001) in the prostatic fluid without significantly (p > 0.5) affecting those of the other parameters determined. The 3H/13C observed in the prostatic fluid following the i.v. injection of DHT-3H and E2-13C was increased following Estracyt, primarily due to a decreased excretion of radioactivity associated with E2. This is in keeping with the lesser amounts of radioactivity of E2 present in the prostatic gland following treatment with Estracyt (see Charts 2 to 4). Thus, the canine prostatic secretion is definitely affected by the Estracyt administered.

Effects of Estracyt on Rat Prostatic Weight and 5α-Reductase and Arginase Activities. In order to determine the antiprostatic effect in the rat, groups of 7 animals were each given various doses of Estracyt i.p. daily for 4 days and the weight of the prostate glands and kidney was determined. The 5α-reductase and arginase activities were also established. The results are shown in Charts 5 to 8. The enzyme results are expressed in 2 ways: per g of tissue and per total gland. It is evident that the weight of ventral and dorsolateral glands decreased significantly, particularly when a very large dose of Estracyt was given (Chart 5). The body weight of the animals did not change, nor was there a significant

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Table 1

Effects of Estracyt on the in vivo deposition of injected testosterone-\(^{3}H\) (20 \(\mu\)Ci) and \(E_{\alpha},^{14}C\) (4 \(\mu\)Ci) in intact and castrated male rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group 1*</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral prostate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^{3}H)</td>
<td>195.1 ± 55.0 (2.3)*</td>
<td>287.8 ± 100.1 (4.4)</td>
<td>174.2 ± 19.4 (3.2)</td>
<td>187.7 ± 25.8 (2.6)</td>
</tr>
<tr>
<td>(^{14}C)</td>
<td>10.9 ± 2.3 (1.8)</td>
<td>23.3 ± (3.0)</td>
<td>7.4 ± 0.3 (1.6)</td>
<td>9.3 ± 0.7 (1.7)</td>
</tr>
<tr>
<td>(^{3}H/^{14}C)</td>
<td>17.5 ± 1.6</td>
<td>18.9 ±</td>
<td>23.5 ± 1.8</td>
<td>21.9 ± 1.7</td>
</tr>
<tr>
<td>Dorsolateral prostate</td>
<td></td>
<td></td>
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<tr>
<td>(^{3}H)</td>
<td>324.2 ± 37.5 (3.9)</td>
<td>310.0 ± 118.0 (4.8)</td>
<td>279.8 ± 44.0 (4.7)</td>
<td>283.0 ± 79.2 (3.6)</td>
</tr>
<tr>
<td>(^{14}C)</td>
<td>20.4 ± 3.5 (3.5)</td>
<td>16.1 ± 6.5 (2.8)</td>
<td>14.2 ± 2.8 (2.9)</td>
<td>12.2 ± 2.8 (2.2)</td>
</tr>
<tr>
<td>(^{3}H/^{14}C)</td>
<td>16.2 ± 1.4</td>
<td>19.5 ± 0.8</td>
<td>19.9 ± 1.3</td>
<td>22.9 ± 1.3</td>
</tr>
<tr>
<td>Seminal vesicles</td>
<td></td>
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</tr>
<tr>
<td>(^{3}H)</td>
<td>196.4 ± 19.2 (2.3)</td>
<td>157.0 ± 19.0 (2.5)</td>
<td>176.6 ± 42.1 (3.0)</td>
<td>181.5 ± 20.8 (2.2)</td>
</tr>
<tr>
<td>(^{14}C)</td>
<td>12.6 ± 2.3 (2.1)</td>
<td>7.7 ± 0.9 (1.4)</td>
<td>8.2 ± 2.4 (1.7)</td>
<td>4.2 ± 2.5 (1.3)</td>
</tr>
<tr>
<td>(^{3}H/^{14}C)</td>
<td>16.0 ± 1.4</td>
<td>20.3 ± 0.4</td>
<td>21.9 ± 1.4</td>
<td>44.1 ± 25.9</td>
</tr>
<tr>
<td>Levator ani</td>
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<tr>
<td>(^{3}H)</td>
<td>103.5 ± 31.3 (1.2)</td>
<td>98.2 ± 3.1 (1.6)</td>
<td>68.2 ± 9.5 (1.2)</td>
<td>109.9 ± 26.2 (1.5)</td>
</tr>
<tr>
<td>(^{14}C)</td>
<td>4.8 ± 0.3 (0.8)</td>
<td>6.8 ± 0.7 (1.3)</td>
<td>4.8 ± 0.4 (1.0)</td>
<td>6.9 ± 1.3 (1.3)</td>
</tr>
<tr>
<td>(^{3}H/^{14}C)</td>
<td>16.1 ± 0.4</td>
<td>14.6 ± 1.0</td>
<td>14.0 ± 0.7</td>
<td>15.7 ± 0.8</td>
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<tr>
<td>Testes</td>
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<tr>
<td>(^{3}H)</td>
<td>153.4 ± 25.1 (1.8)</td>
<td>95.2 ± 13.8 (1.6)</td>
<td>8.6 ± 1.0 (1.8)</td>
<td>11.0 ± 0.6</td>
</tr>
<tr>
<td>(^{14}C)</td>
<td>11.2 ± 1.8</td>
<td>11.0 ± 0.2</td>
<td>11.0 ± 0.2</td>
<td>11.0 ± 0.6</td>
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<tr>
<td>(^{3}H/^{14}C)</td>
<td></td>
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<tr>
<td>Pancreas</td>
<td></td>
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<tr>
<td>(^{3}H)</td>
<td>351.6 ± 65.4 (4.2)</td>
<td>165.8 ± 54.2 (2.4)</td>
<td>141.1 ± 12.9 (2.4)</td>
<td>176.8 ± 26.6 (3.0)</td>
</tr>
<tr>
<td>(^{14}C)</td>
<td>83.7 ± 36.3 (13.9)</td>
<td>43.6 ± 16.2 (7.1)</td>
<td>46.2 ± 4.3 (9.9)</td>
<td>37.7 ± 26.4 (9.0)</td>
</tr>
<tr>
<td>(^{3}H/^{14}C)</td>
<td>5.1 ± 1.6</td>
<td>3.9 ± 0.4</td>
<td>3.1 ± 0.2</td>
<td>3.8 ± 0.8</td>
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<tr>
<td>Kidney</td>
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<tr>
<td>(^{3}H)</td>
<td>369.2 ± 57.1 (4.4)</td>
<td>302.5 ± 58.3 (4.7)</td>
<td>211.5 ± 19.8 (3.6)</td>
<td>294.3 ± 22.8 (4.0)</td>
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<tr>
<td>(^{14}C)</td>
<td>21.8 ± 4.8 (3.6)</td>
<td>18.2 ± 5.2 (2.2)</td>
<td>13.0 ± 0.4 (2.8)</td>
<td>13.5 ± 1.5 (2.6)</td>
</tr>
<tr>
<td>(^{3}H/^{14}C)</td>
<td>17.3 ± 1.0</td>
<td>17.7 ± 2.4</td>
<td>16.3 ± 1.1</td>
<td>21.9 ± 1.1</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
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</tr>
<tr>
<td>(^{3}H)</td>
<td>84.2 ± 12.4</td>
<td>65.7 ± 14.7</td>
<td>58.9 ± 19.0</td>
<td>75.1 ± 13.3</td>
</tr>
<tr>
<td>(^{14}C)</td>
<td>6.0 ± 0.4</td>
<td>5.8 ± 1.6</td>
<td>4.8 ± 0.7</td>
<td>4.1 ± 2.4</td>
</tr>
<tr>
<td>(^{3}H/^{14}C)</td>
<td>14.0 ± 0.5</td>
<td>11.7 ± 1.3</td>
<td>1.4 ± 1.6</td>
<td>13.9 ± 0.3</td>
</tr>
</tbody>
</table>

* Group 1, control intact; Group 2, control castrated; Group 3, intact pretreated with Estracyt; Group 4, castrated pretreated with Estracyt.

Numbers in parentheses, ratios of dpm in a g of tissue to those in a g of muscle.

Table 2

Prostatic fluid composition before and after treatment of dogs with Estracyt

<table>
<thead>
<tr>
<th></th>
<th>Av. values of determinations in 2 dogs (shown in parentheses)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloride (mEq/l)</td>
</tr>
<tr>
<td>Before treatment</td>
<td>114.0 (108.0, 120.0)</td>
</tr>
<tr>
<td>After treatment</td>
<td>136.0 (135.1, 136.9)</td>
</tr>
</tbody>
</table>

change in the weight of the kidney. The latter organ was included, since it has considerable 5α-reductase activities, subject to control by androgens and possibly by estrogens. There was some reduction in testicular weight (pre-Estracyt 1745 ± 21 mg versus 1594 ± 78 post-Estracyt). The 5α-reductase and arginase activities declined significantly in the prostatic glands, particularly in the ventral one (Charts 6 to 8). No change in the 5α-reductase activity was observed in the kidney, although a decrease in the arginase activity was found.

Effect of Estracyt on 5α-Reductase of Dog Prostate.
The 5α-reductase in the prostate of dogs pretreated with Estracyt (5 mg/day i.v. for 2.5 days) was determined in 3 animals. The canine prostatic 5α-reductase usually ranges between 4 to 8% in control animals in our laboratory. The values found in the 3 dogs treated with Estracyt were 0.25,
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Chart 5. Weight of prostate glands and kidney in rats treated with various doses of Estracyt. The results are expressed as a percentage of control values. Each point consists of the average of the values obtained on 7 rat prostates. Only a slight decrease in the weight of the kidney was evident, whereas the 2 prostates showed significant decreases in weight, particularly the ventral gland.

Chart 6. Arginase activity, expressed per g of tissue, in rat prostates and kidney following the administration of various doses of Estracyt. In the dorsolateral gland, except for a decrease at the lowest dose, the arginase activity was increased with the higher doses. The decreased activities in the ventral gland and kidney, although not very profound, were more consistent.

1.4, and 2.8%. These decreases are probably significant. It is possible that further decreases would have been obtained upon prolonged Estracyt therapy.

DISCUSSION

The increasing importance of combinations of chemotherapeutic agents, including chemical conjugates, with steroid hormones in human cancer warrants some discussion regarding the data obtained with Estracyt. The results obtained with Estracyt in 1 animal and/or its tissues should not be extrapolated regarding its action or effectiveness in another. In one animal the Estracyt may be readily hydrolyzed totally before it reaches the prostate, whereas in another it may be but insignificantly hydrolyzed, e.g., in the human (A. A. Sandberg et al., unpublished observation). Hence, the results presented in the present paper apply to the dog and rat and should be extrapolated to the human or other animals with great caution. Thus, we have found that the metabolism and fate of labeled Estracyt in the human is quite different from that of E2 and indicates minor hydrolysis of the Estracyt molecule. The results in the dog and baboon are significantly different from each other and from those in man.

A double nitrogen mustard ester of E2 (NSC 11259), i.e., alkylating agents attached at both the 3 and 17 positions of this steroid, has been shown to have antitumor activity in a number of experimental tumors in rats (23, 24). Since E2 per se was not effective in all the tumor systems studied, whereas the E2 mustard was, it was inferred that antitumor activity must have originated in the mustard moiety, even though definite estrogenic effects were seen in the animals. The toxicity of the mustard alone was much greater than that of the E2 mustard. A number of severe toxic symptoms was observed in beagles given this E2 mustard (17) and these apparently precluded its use in humans. This toxicity contrasts with the lack of such following Estracyt administration to humans (11–13) and may indicate that the

Chart 7. Arginase activity expressed in terms of total organ in the prostate glands and kidney of rats treated with various doses of Estracyt. In the case of the ventral prostate and kidney, the combination of decreased weight and decreased arginase activity led to a very significant decrease in total organ activity. In the case of the dorsolateral gland, the effects are not very consistent and present only at the lowest dose of Estracyt.

Chart 8. The effects of various doses of Estracyt on 5α-reductase activity of rat prostates. The decrease in the activity of the ventral prostate is profound but is less so in the dorsolateral gland. These values, taken in conjunction with the weight reduction of the glands (see Chart 5), indicate very greatly decreased 5α-reductase activity in the prostates of the rat. The kidney did not show this decrease and, if anything, 5α-reductase activity was elevated by the administered Estracyt.
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nitrogen mustard moieties of the former drug (NSC 11259) become readily hydrolyzed and capable of producing systemic effects. This is further substantiated by the evident estrogenic effects, including that on the prostate, observed in the animals treated with the double ester of E₂ (17).

The data of the present study indicate that Estracyt has a definite antiprostatic effect as manifested by the decreased weight of the gland in the rat; decreased prostatic secretion in the dog; and the decreased levels of acid phosphatase, 5α-reductase, and arginase activities in the dog and rat prostates. Since only some of these effects and/or a combination of these can be produced by the administration of E₂ or other estrogens to intact animals, and others may be due to the nitrogen mustard moiety, the data point to a rather complicated mechanism of antiprostatic action of Estracyt. Thus, if in fact some of the antiprostatic effects are due to the E₂ moiety, these indicate a capacity on the part of the prostate to hydrolyze in situ both the phosphate and nitrogen mustard moieties, since only the free E₂ is biologically effective. It is possible that such hydrolyses occurred in the body at a site other than the prostate and that the E₂ thus released is then available for the production of the antiprostatic effects observed either through inhibition of interstitial cell-stimulating hormone release from the pituitary and/or direct effect on testosterone secretion by the testes, or testosterone effects in the prostate per se. This indeed remains a distinct possibility, at least in some animals, although there is suggestive evidence in the present study that the phosphate may remain attached to the E₂ and subsequently be hydrolyzed in the prostate. This is borne out by the failure of Estracyt or E₂-phosphate to affect the deposition of E₂ or E₃ in the pancreas of the dog, while affecting the uptake in the prostate. It appears, thus, that dephosphorylation may be a key step in the effects of Estracyt and that such hydrolysis is readily accomplished by the prostates of the dog and rat.

Were a major part of the E₂ in Estracyt to be released at a site from which it could become systemically effective, one would expect to see major estrogenic effects in organs and tissues other than the prostate. This has not been the case in humans (11–13) or mice (J. Müntzing et al., unpublished observation), and the observations indicate that if Estracyt is hydrolyzed at a site other than the prostate it does not leave that site in a form that is biologically effective.

It has been demonstrated by Tritsch et al. (19) that human serum phosphatases readily hydrolyze off the phosphate group of Estracyt. The drug was shown to have a high affinity for the enzymes, particularly for alkaline phosphatase, but the highest reaction velocity was observed with acid phosphatase.

Some of the observations obtained point to the nitrogen mustard of Estracyt as playing a part in the antiprostatic effects observed. The interference of Estracyt with the uptake of DHT by the rat prostate and the reduction of 5α-reductase activity in the prostates of the dog and rat indicate a possible direct effect of the nitrogen mustard, since such a combination of effects has not been observed with estrogens alone.

ACKNOWLEDGMENTS

We wish to thank Dr. H. Yamanaka for the arginase determinations; Dr. T. M. Chu for the chemistries on the prostatic fluids; Christine Hanson for clerical help; and Joyce Romano, Anne Agazz, Carol Housten, and Elek Karsay for technical assistance.

REFERENCES

Antiprostatic Action of Estracyt


