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# Suppression of Progestational Uterine Mucosal Activity by Anthrarufin*

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The effect of anthraquinone derivatives (anthraquinone, sodium anthraquinone-$\beta$-sulfonate, anthrarufin and anthrone) and other compounds ($\alpha$-naphthoquinone, lecithin and tannic acid) on progestational proliferation produced by progesterone in the rabbit uterus was investigated by measurements of endometrial carbonic anhydrase activity.

In immature rabbits primed with estrogen, the subcutaneous administration of progesterone (2 mg/animal for 5 days) caused a typical activation of endometrial carbonic anhydrase. Of the test compounds used, oral treatment with anthrarufin in company with progesterone inhibited the enzyme activation by progesterone. The most effective dose was 1.0 mg, causing a 50% inhibition to the action of 2 mg progesterone. When given subcutaneously together with progesterone, anthrarufin caused little or no inhibition of the progesterone-induced enzyme activation. Oral administration of anthraquinone, sodium anthraquinone-$\beta$-sulfonate, $\alpha$-naphthoquinone, lecithin and tannic acid did not interfere with the endometrial response to progesterone. It thus appears that in the rabbit oral administration of anthrarufin causes a significant inhibition of the progesterone-induced increase in endometrial carbonic anhydrase activity and has an antiprogestational action.

In the course of our studies for finding certain plant preparations having antiprogestational activity, Kurouji[1] found that an aqueous extract of *Radix polygoni multiflori* (*Polygonium multiflorum* THUNBERG), when given orally at the time of a subcutaneous injection of progesterone, could prevent the augmented activity of endometrial carbonic anhydrase produced by progesterone. This suggests that the extract contains some materials against progestational activity, because the enzyme activity increases roughly in proportion to proliferative development of the uterine mucosa.[2-4] In view of the result of Igasa and Tatsumi[5] that *Radix polygoni multiflori* contained oxymethylanthraquinone derivatives, lecithin and others, it seemed important to investigate the antagonistic action of anthraquinone derivatives and some compounds. The following compounds were used: anthraquinone, sodium anthraquinone-$\beta$-sulfonate, anthrarufin, anthrone, $\alpha$-naphthoquinone, lecithin and tannic acid.

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## Methods

The methods used were identical with those described previously.[6,7] Immature albino rabbits weighing approximately 1.5 kg were used. Clauberg's method[8] was modified in that the animals were primed with estradiol-17$\beta$ and received a standard dose of 2 mg progesterone; simultaneously, one of the test compounds was administered by a stomach tube in logarithmically graded doses of 0.001–1,000 mg/animal. The test compounds were also administered subcutaneously as needed. Twenty-four hours after the last administration, the animals were killed by exsanguination through the carotid artery under cervical dislocation. The uteri were excised, and the endometrium was dissected and homogenized with cold distilled water. The homogenate was centrifuged at 2,000 rpm for 10 minutes and supernatant was analyzed for the activity of carbonic anhydrase by the method of Miyake and Pincus;[9] the activity was expressed in terms of enzyme units/g wet weight of the endometrium.[10]

In experiments with anthrarufin, histological examination was carried out to compare the enzyme response and progestational proliferation. A representative cross section of the uterus was stained by hematoxylin-eosin, projected and traced. The glandular and total mucosal areas were planimetrically measured, and the ratio of the former to the latter (G/M ratio) was calculated; the ratio served as an index of progestational proliferation.

*Administrations of chemicals.* The following chemicals were used: estradiol-17$\beta$ (Sigma Co.), progesterone (Sigma Co.), anthraquinone (Wakô Co.), sodium anthraquinone-$\beta$-sulfonate (Wakô Co.), anthrarufin (Tokyo Kasei Co.), anthrone (Wakô Co.) and tannic acid (Wakô Co.). Estradiol-17$\beta$ dissolved in sesame oil was administered into the neck in a dose of 5 $\mu$g once daily for 6 days, the injection volume being 0.1 ml/animal daily. Total doses of 0.5 to 2.0 mg of progesterone, dissolved in sesame oil, were given in 5 divided doses at daily intervals into the neck for 5 days, the injection volume being 0.2 ml/animal daily. Anthrarufin (0.001–10.0 mg) in a thin alkaline saline solution (pH=7.1–8.8), sodium anthraquinone-$\beta$-sulfonate (0.01–10.0 mg) and tannic acid (0.1–1,000 mg) in 0.9% NaCl solution, anthraquinone (0.1–1,000 mg), anthrone (0.01–100 mg) and $\alpha$-naphthoquinone (0.01–100 mg) in a saline suspension, and lecithin (0.01–100 mg) in a colloidal suspension in saline solution were administered by a stomach tube simultaneously with a subcutaneous injection of progesterone once daily for 5 days, and the daily volume was 3 ml/animal.

## Results and Remarks

### *Activation of endometrial carbonic anhydrase in response to progesterone*

When progesterone was administered subcutaneously in total doses of 0.5–2.0 mg/animal to groups of 5–6 rabbits primed with estrogen, an unequivocal increase in the activity of endometrial carbonic anhydrase was observed. The increase of the activity was dose-dependent. The degree of response was directly correlated to the logarithm of progesterone doses in a nearly linear pattern (Table 1). In a histological G/M ratio of the uterus, the relationship between the logarithm of the dose and the degree of response was similar to the result from enzyme determination. These findings agree with the results of the other workers.[3,4,11]

### *Influence of the test compounds on the activating effect of progesterone on the endometrial carbonic anhydrase*

The experiments were performed for the purpose of evaluating the inhibitory effect of the test compound on the endometrial carbonic anhydrase concentration at a standard dose of progesterone. Two mg of progesterone were used as the standard dose.

TABLE 1. *Carbonic anhydrase activity and G/M ratio in the rabbit endometrium stimulated with progesterone*

| Dose (mg/animal) of progesterone | No. of animal | Carbonic anhydrase content (unit/g tissue)* | G/M ratio of uterus |
|---|---|---|---|
| 0 | 5 | 31± 3 | 0.41±0.02 |
| 0.5 | 6 | 123±12 | 0.52±0.04 |
| 1.0 | 6 | 311±26 | 0.61±0.05 |
| 1.5 | 5 | 408±34 | 0.70±0.05 |
| 2.0 | 6 | 515±32 | 0.73±0.03 |

* Means±S.E.
G/M: Glandular area divided by total mucosa area in uterine section.

*Anthrarufin*

In control rabbits, treatment with 2 mg of progesterone produced a typical activation of endometrial carbonic anhydrase; the endometrium of these animals showed enzyme values of 498±33 EU/g with a histological G/M ratio of 0.72± 0.05. With oral administration of 0.001–1.0 mg (with the exception of 0.01 mg) of anthrarufin together with progesterone, a significant inhibition of the enzyme activation characteristic of progesterone was found as shown in Table 2; 30% inhibition with 0.001 mg, 38% inhibition with 0.1 mg and 50% inhibition with 1.0 mg ($P<0.01$). Histological examination of the endometrium in these groups showed a G/M ratio of 0.70±0.06, 0.70±0.03 and 0.57±0.04, respectively, and that of 1.0 mg was distinctly below that of the endometrial activity stimulated by progesterone alone. In doses over 1.0 mg, there was no significant difference in the response of the endometrial enzyme to progesterone with or without anthrarufin. In other experiments with subcutaneous administration, anthrarufin did not essentially interfere with the endometrial response to progesterone. Doses of 0.001–0.01 mg were thought to have a slight effect, but the response appeared erratic in that the extent of activity in the endometrial enzyme showed a great variability (Table 3). Moreover, when anthrarufin alone was given either orally or subcutaneously, no significant change in the endometrial enzyme activity was observed as compared with those in the absence of anthrarufin (Table 2).

*Anthraquinone, sodium anthraquinone-β-sulfonate, anthrone, α-naphthoquinone, lecithin and tannic acid*

Anthraquinone, sodium anthraquinone-β-sulfonate and anthrone did not counteract the luteoid activity of progesterone, and the degree of enzyme activity was essentially the same as that of the enzyme activation by progesterone alone. α-Naphthoquinone and lecithin were also examined, and the result was negative. Tannic acid did not show any marked inhibition on progestation except that 10 mg administration showed a slight effect and caused a 27% inhibition.

The experimental results in this study reveal that, of several compounds used, anthrarufin only antagonizes the action of progesterone on the rabbit endo-

TABLE 2. *Effect of oral administration of anthrarufin on the increase of endometrial carbonic anhydrase produced by progesterone*

| Dose (mg/animal) | | No. of animals | Carbonic anhydrase content (unit/g tissue)* | G/M ratio of uterus | % inhibition of progesterone by anthrarufin (enzyme test) |
|---|---|---|---|---|---|
| Progesterone | Anthrarufin | | | | |
| 0 | 0 | 6 | 32±4 | 0.42±0.02 | |
| 2.0 | 0 | 9 | 498±33 | 0.72±0.05 | |
| 2.0 | 10.0 | 6 | 469±46 | 0.75±0.07 | — |
| 2.0 | 1.0 | 9 | 265±21† | 0.57±0.04 | 50 |
| 2.0 | 0.1 | 13 | 323±32† | 0.70±0.03 | 38 |
| 2.0 | 0.01 | 7 | 437±54 | 0.72±0.06 | — |
| 2.0 | 0.001 | 6 | 356±39† | 0.70±0.06 | 30 |
| 0 | 10.0 | 4 | 33±8 | | |
| 0 | 1.0 | 5 | 27±4 | | |
| 0 | 0.1 | 5 | 31±7 | | |

* Mean±S.E.

† $P<0.01$ compared with enzyme response to 2 mg progesterone alone.

G/M: Glandular area divided by total mucosa area in uterine section.

Percentage inhibition is expressed as $\frac{Rp-Ra}{Rp-C} \times 100$; Rp=uterine enzyme response to progesterone alone; Ra, uterine enzyme response to combination of progesterone and anthrarufin; C, control level.

TABLE 3. *Effect of subcutaneous administration of anthrarufin on the increase of endometrial carbonic anhydrase produced by progesterone*

| Dose (mg/animal) | | Number of animals | Carbonic anhydrase content (unit/g tissue)* |
|---|---|---|---|
| Progesterone | Anthrarufin | | |
| 0 | 0 | 6 | 31±4 |
| 2.0 | 0 | 8 | 501±32 |
| 2.0 | 1.0 | 7 | 454±48 |
| 2.0 | 0.1 | 7 | 523±59 |
| 2.0 | 0.01 | 8 | 370±40 |
| 2.0 | 0.001 | 8 | 386±46 |

* Means±S.E.

metrium. It was potentially active on oral administration, but little or not active on subcutaneous administration. The most effective dose in oral administration was 1.0 mg/animal, causing a 50% inhibition to the action of 2 mg progesterone.

Previously Kurouji[1] working with almost the same procedure showed that the oral administration of an aqueous extract of *Radix polygoni multiflori* inhibited the activating effect of progesterone on the endometrial carbonic anhydrase. In his experiments, the most effective dose was 100 mg/animal against the action of 2 mg progesterone and the effect of progesterone was reduced by 56%. However, when given subcutaneously, polygonium extracts failed to influence the progesterone-induced enzyme activation. Analytical studies of *Radix polygoni multiflori* by Igasa and Tatsumi[5] is of particular interest in that it contains principally

oxymethylanthraquinone derivatives in 1.78%. It would therefore be expected that the inhibitory action is due to oxymethylanthraquinone derivatives. However, we have not yet tested this compound as a progestational inhibitor in the rabbit. In the present study, anthrarufin, a derivative related to anthraquinone, showed an antiprogestational action, although anthraquinone and anthrone were inactive. In this respect, the hydroxyl structure of anthrarufin is needed for the response and may exhibit the inhibitory activity on the endometrial progestation. The question arises whether anthrarufin itself is *in vivo* a potent progestational inhibitor on injection. It seems likely that *in vivo* conversion product of anthrarufin is responsible for the progestational inhibition and presumably this conversion takes place in the guts or in the liver. The present study, however, does not permit any explanation as to the site of anthrarufin action and the mechanism of this antagonism. It was not investigated whether the action of this substance is a direct one on the endometrium or is due to neutralization or inactivation by anthrarufin of progesterone present in the body.

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