Progesterone Antagonism of Papain Emphysema: Role of Sex, Estrogens and Serum Antitrypsin (37619)

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Emphysema-like lesions have been produced in several species by intratracheal or aerosol administration of papain (1–6). Progesterone has been reported to prevent the emphysema-like lesions produced in rats by papain and tracheal ligation (7) or by phytohemagglutinin and tracheal ligation (8). Earlier studies in this laboratory indicated that progesterone protected male rats, but not females, from papain aerosol-induced emphysema (9).

The mechanism of the protective action of progesterone is unknown. A deficiency of serum α_1-antitrypsin has been related to human panlobular emphysema (10). Serum antitrypsin activity is elevated in pregnancy (11–13) and in women taking oral contraceptive medication containing progesterone and mestranol (13).

The following studies using papain aerosol-induced emphysema were undertaken to (a) confirm the sex difference in response to progesterone under identical experimental conditions; (b) evaluate the interaction of estrogens and progesterone in male rats; (c) evaluate the effect of progesterone in ovariectomized rats; and (d) study the effect of progesterone and estrogen on serum antitrypsin activity as a possible mechanism for their actions in this model.

Materials and Methods. Treatment of animals. Sprague-Dawley rats (5–7 weeks old) were used in these studies. Ovariectomies were performed 12 days prior to initiation of papain administration. Papain[1] aerosol was administered as previously described (4) as a 3% solution in water delivered by four nebulizers into a closed Plexiglas chamber (34 × 21 × 19 in.) containing the rats. The nebulizers were operated at a pressure of 10 lb/in.[2] Four cages of rats (5/cage) were placed in the chamber and given a 1 hr papain aerosol treatment three times a week for a 4 wk period.

Evaluation procedures. Severity of the emphysema was evaluated by measurements of functional residual capacity (FRC) by a modification of the method of King (14) and by an objective air space rating system. At the end of the 4 wk period, both control and papain-treated rats were anesthetized with Dial–urethane (0.5 ml/kg, ip)[2] and connected by a short tracheotomy tube to a 10 ml syringe containing pure oxygen. The connection was made at the end of expiration and the animals then respired in this closed system for 7 min. Carbon dioxide was removed by an 11% solution of potassium hydroxide in the bottom of the "oxygen syringe" and oxygen was bubbled into the syringe to replace the carbon dioxide. The rat was disconnected from the syringe at the end of expiration and the syringe closed to atmosphere. The percentage nitrogen in the syringe was determined by use of an oxygen analyzer (Instrument Laboratory), *i.e.*, 100% — % oxygen = % nitrogen. Nitrogen concentration in the "oxygen syringe" was converted to FRC (ml) by means of a calibration curve previously obtained by equilibrating known volumes of air with the apparatus.

Following FRC determinations, rats from certain groups were decapitated and blood samples were collected. Blood was allowed to clot for 1 hr after centrifugation and serum was removed and stored at —20° until assayed for antitrypsin activity according to

[1] Supplied by Warner-Chilcott Laboratories.

[2] 100 mg of allobarbital, 400 mg of urethane and 400 mg of monoethylurea in each ml.

the method of Ericksson (15). This assay utilizes benzoyl-DL-arginine-*p*-nitroanilide hydrochloride (BAPNA) as a trypsin substrate. Trypsin was standardized by titration with soybean trypsin inhibitor (STI). STI produced a linear inhibition of trypsin activity with a trypsin/STI combining ratio of 1.3:1. Preincubation of trypsin with increasing amounts of serum resulted in a linear inhibition of trypsin activity, until approximately 80% inhibition was achieved. All values reported were obtained from determinations on the linear portion of the inhibition curve. Values are expressed as milligrams of trypsin per milliliter of serum (trypsin inhibitory capacity or TIC). Previous work had indicated that neither Dial–urethane nor freezing had any effect on serum TIC. TIC values were also determined from serums of certain rats sacrificed 10 or 24 days after initiation of treatments; no other measurements were made on these animals.

Following decapitation, the thorax of the rat was opened and the lungs were distended with buffered neutral formalin fixation fluid at a pressure of 10 cm H_2O and removed intact. Lungs were left in the fixative until sectioned. After fixation, 5 μm sections were cut from paraffin blocks and stained with hematoxylin and eosin.

For objective determinations of lung damage, a grid with 30 cross marks 12 mm apart (6 × 5 rows) was placed over the viewing screen of a Unitron microscope. Slides were examined at a magnification of 150×. Eight fields were randomly chosen from a single lung slide of the right lower lobe. Lung spaces were counted in the following manner: if the cross marks fell on alveolar septa, blood vessels, connective tissue or any large air vessel wall, no score was given; if the cross mark fell on an empty space, a rating of one was given. Thus, a maximum of 240 points was possible. An increase in air space would indicate either a loss of tissue, enlarged air space or both. This counting system was adapted from the work of Dunnill (16) and Palecek, Palecekova and Aviado (2).

Statistical evaluation. Differences between means of two treatment groups were determined using Student's *t* test.

Drugs. Progesterone, estradiol-17β and diethylstilbestrol were administered sc in sesame oil. Injections were made 5 times/wk during the 4 wk of papain administration. Salt free trypsin, twice crystallized and lyophilized, salt free and lyophilized STI, BAPNA and estradiol-17β were obtained from Mann Research Laboratories. Progesterone was obtained from Calbiochem; diethylstilbestrol

TABLE I. Effect of Progesterone on Papain Emphysema in Male and Female Rats.

Drug treatment[a]	Sex	Wt (g)	Functional residual capacity (ml)	Functional residual capacity (ml/kg)	Air spaces
Untreated controls	M	247.3 ± 14.3[b] (21)[c]	2.63 ± 0.14 (21)	10.28 ± 0.42 (21)	47.1 ± 1.7 (10)
None	M	246.9 ± 9.2 (11)	3.64 ± 0.21[d] (11)	14.67 ± 0.76[d] (11)	64.3 ± 2.4[d] (11)
Progesterone (5 mg/kg sc)	M	213.3 ± 8.8 (8)	2.42 ± 0.14 (8)	11.83 ± 0.14 (8)	45.7 ± 3.2 (8)
Untreated controls	F	187.5 ± 7.8 (22)	2.41 ± 0.10 (22)	12.72 ± 0.44 (22)	48.4 ± 7.9 (11)
None	F	201.3 ± 6.2 (27)	3.56 ± 0.18[d] (23)	17.92 ± 0.87[d] (23)	66.2 ± 2.8[d] (17)
Progesterone (5 mg/kg sc)	F	210.0 ± 6.3 (12)	3.18 ± 0.17[d] (6)	15.85 ± 0.73[d] (6)	60.0 ± 2.9[d] (12)

[a] All groups, except untreated controls, were treated with papain aerosol.
[b] Mean ± SE.
[c] Number of values in sample.
[d] Significantly different from untreated control of same sex, $p < 0.05$.

TABLE II. The Influence of Selected Estrogens and Progesterone on Papain Emphysema in Male Rats.

Drug treatment[a]	Daily dose (sc)	Wt (g)	Functional residual capacity (ml)	Functional residual capacity (ml/kg)	Air spaces
Untreated controls	—	279.7 ± 8.4[b] (19)[c]	2.32 ± 0.14 (19)	8.79 ± 0.40 (19)	48.8 ± 3.3 (10)
None	—	283.1 ± 11.9 (8)	3.59 ± 0.36[d] (6)	13.28 ± 1.30[d] (6)	72.0 ± 6.6[d] (9)
Progesterone	5 mg/kg	273.6 ± 10.2 (12)	2.33 ± 0.13 (7)	8.61 ± 0.56 (7)	50.5 ± 1.9 (12)
Estradiol-17β	1 μg/rat	272.1 ± 13.6 (10)	3.57 ± 0.46[d] (7)	12.89 ± 1.78[d] (7)	64.5 ± 4.7[d] (10)
Diethylstilbestrol	1 μg/rat	209.3 ± 12.6[d] (19)	2.66 ± 0.70 (14)	13.69 ± 1.77[d] (14)	61.3 ± 3.1[d] (19)
Progesterone + estradiol-17β	5 mg/kg + 1 μg/rat	258.2 ± 10.6 (12)	3.40 ± 0.54[d] (7)	13.17 ± 2.37[d] (7)	65.3 ± 3.5[d] (12)
Progesterone + diethylstilbestrol	5 mg/kg + 1 μg/rat	239.9 ± 9.9[d] (9)	3.10 ± 0.37[d] (6)	14.05 ± 1.68[d] (6)	68.2 ± 5.4[d] (9)

[a] All groups, except untreated controls, were treated with papain aerosol.
[b] Mean ± SE.
[c] Number of rats in sample.
[d] Significantly different from untreated control group, $p < 0.05$.

was supplied by Merck.

Results. Comparison of the effects of progesterone on papain emphysema in male and female rats. The effect of progesterone on papain-challenged rats is shown in Table I. Progesterone protected the males, but not the females, from papain-induced changes in FRC and air spaces.

Effect of estrogen on the protective action of progesterone in papain challenged males. The effect of progesterone on estrogen-treated male rats is shown in Table II. Both estradiol-17β and diethylstilbestrol prevented the protective effect of progesterone. Neither of the estrogens alone influenced the effect of papain. The FRC (ml) value for papain-treated rats that received diethylstilbestrol was not significantly elevated from control, but the weight of the former group was significantly lower than control and the FRC (ml/kg) and air space rating was significantly elevated, $p < 0.05$.

Effect of progesterone on papain emphysema in ovariectomized rats. The effect of progesterone on ovariectomized rats treated with papain is shown in Table III. Progester-

TABLE III. Effect of Progesterone on Papain Emphysema in Ovariectomized Rats.

Drug treatment[a]	Wt (g)	Functional residual capacity (ml)	Functional residual capacity (ml/kg)	Air spaces
Untreated controls	275.0 ± 15.6[b] (9)[c]	2.51 ± 0.24 (9)	8.95 ± 0.66 (9)	49.2 ± 2.3 (13)
None	270.0 ± 9.4 (10)	3.53 ± 0.37[d] (10)	12.97 ± 1.42[d] (10)	66.7 ± 3.1[d] (7)
Progesterone 5 mg/kg sc	256.1 ± 14.1 (9)	2.34 ± 0.29 (9)	9.1 ± 0.88 (9)	52.6 ± 3.8 (7)

[a] All groups, except untreated controls, were treated with papain aerosol.
[b] Mean ± SE.
[c] Number of values in sample.
[d] Significantly different from untreated controls, $p < 0.05$.

TABLE IV. The Effect of Various Treatments on Serum Antitrypsin Activity in Rats.

Drug treatment[a]	Daily dose (sc)	Trypsin inhibitory capacity[b]		
		Day 10[d]	Day 24[d]	Days 29–30[d,e]
Untreated controls	—	2.53 ± 0.26[c]	2.60 ± 0.20	2.76 ± 0.11
None	—	2.62 ± 0.25	2.44 ± 0.20	2.85 ± 0.18
Progesterone	5 mg/kg	2.38 ± 0.16	2.48 ± 0.17	2.95 ± 0.15
Estradiol-17β	1 μg/rat	2.62 ± 0.25	2.88 ± 0.08	2.47 ± 0.22
Diethylstilbestrol	1 μg/rat	2.50 ± 0.16	2.60 ± 0.13	2.51 ± 0.13
Progesterone + estradiol-17β	5 mg/kg + 1 μg/rat	2.28 ± 0.19	2.63 ± 0.23	2.49 ± 0.24
Progesterone + diethylstilbestrol	5 mg/kg + 1 μg/rat	2.40 ± 0.19	2.70 ± 0.11	2.51 ± 0.14

[a] All groups, except untreated controls, were treated with papain aerosol.
[b] Mg trypsin inhibited/ml serum.
[c] Each value is mean ± SE for 7–10 animals.
[d] Papain and drug treatments were started on Day 1.
[e] FRC and air space values for animals sacrificed on these days found in Table II.

one prevented the papain response in these animals.

Effect on serum trypsin inhibitory activity. The effects of progesterone, estrogen or papain and their combinations on serum trypsin inhibitory capacity are shown in Table IV. None of the treatments affected serum trypsin inhibitory capacity measured during, or at the completion of the treatment schedule.

Discussion. The ability of progesterone to protect rats from papain-induced emphysema is antagonized by estrogens. Both estradiol-17β and diethylstilbestrol prevented the beneficial effect of progesterone in male rats. Progesterone had no protective effect in normal females but it did protect ovariectomized females from papain emphysema.

The mechanism of the estrogen-induced antagonism of the protective effect of progesterone is unknown. Neither estrogenic substance by itself influenced the severity of the lesion. It is unlikely that papain aerosol produced unsurmountable changes in FRC and air space values, since higher values than those in the present work have been reported (17). Therefore, it appears unlikely that the antiprogesterone effects of the estrogens were mediated through an exacerbation of the disease. The animals that received diethylstilbestrol lost weight, suggesting that a toxic effects of this drug might contribute to the antagonism of progesterone. However, estradiol-17β caused no weight loss, but antagonized the effect of progesterone.

Estrogen did not alter serum antitrypsin activity indicating a lack of relationship between estrogen-induced effects and serum antiprotease activity. It is interesting that Stabler and Ungar (18) reported that estradiol-17β (administered to male mice and pigs) induced the adrenal activity of 20α-hydroxysteroid dehydrogenase, an enzyme which converts progesterone to a weaker progestational agent, 20α-hydroxyprogesterone (19–22). It is possible that increased hydroxylation of progesterone may be the cause of the estrogen-induced antagonism of progesterone.

The two estrogenic drugs, by themselves, exerted no beneficial effects against papain emphysema. It should be noted that while rats treated with papain and diethylstilbestrol had elevated FRC values based on body weight, the absolute FRC measurements were not increased from control. Diethylstilbestrol decreased body weight. Previous studies had indicated that FRC values and animal weight are significantly and directly related in untreated (4, 14) and in papain-treated rats (4), but no correlation between FRC and weight was found in rats treated with papain and diethylstilbestrol in the present study. Data from air space evaluations indicated that diethylstilbestrol had no beneficial action against papain emphysema.

The mechanism of the beneficial effects of

progesterone on papain-induced emphysema remains obscure. A homozygous deficiency of serum α_1-antitrypsin has been reported to be associated with panlobular emphysema in humans (10). A heterozygous deficiency of antitrypsin has been similarly implicated by some (23–24) and challenged by others (25). Elevated serum antitrypsin levels have been reported in women taking oral contraceptive medication (13) and in pregnancy (11–13). The present experiments, however, showed no relationship between the serum antiprotease activity evaluated during and at the completion of the papain treatment period and the effect of progesterone.

It is therefore unlikely that the beneficial effects of progesterone were mediated through changes in this serum factor.

Summary. An emphysema-like lesion was produced in rats by periodic administration of papain aerosol. Progesterone (5 mg/kg/day) prevented the condition in males and ovariectomized females, but not in unaltered females. Estradiol-17β or diethylstilbestrol, at doses of 1 μg/rat/day, inhibited the protective action of progesterone. Neither of these estrogens alone had protective or deleterious effects in papain-treated rats. None of the hormones studied altered serum antitrypsin activity. Therefore, it is unlikely that either the beneficial effects of progesterone or the antagonistic effects of estradiol-17β or diethylstilbestrol were mediated through changes in this serum factor.

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