

## ANTI-INFLAMMATORY ACTION OF PROGESTERONE ON CARRAGEENIN-INDUCED INFLAMMATION IN RATS

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**Abstract**—Effect of progesterone (1 mg/kg) on the inflammation in rats induced by carrageenin was studied. Progesterone inhibited the vascular permeability and the formation of granulation tissue in the early phase of the inflammation. In the chronic proliferative phase, progesterone suppressed the vascular permeability and there was an active resorption of the granulation tissue. Increased degradation of noncollagen protein was observed in the treated group without apparent influence on the metabolism of collagen or on the synthesis of noncollagen protein. The mode of action of progesterone was compared with that of a potent anti-inflammatory steroid, hydrocortisone acetate.

A temporary remission of inflammatory symptoms in rheumatoid arthritis frequently occurs during pregnancy (1). Concerning the mechanism for such a remission, possible changes in hormonal secretion in the body have to be considered. It is well known that progesterone levels in the serum and tissues are elevated during pregnancy and then fall precipitously in the postpartum period. Lindhe and Sonesson (2) reported that progesterone exerted an inhibitory influence on acute inflammatory process induced by croton oil, while Nyman (3) demonstrated that progesterone increased the vascularity of a wounded area in oophorectomized rabbit. It is important, therefore, to determine how progesterone influences the entire course of acute and chronic phases of the inflammatory processes. The present experiment was undertaken in an attempt to determine the influence of progesterone on carrageenin-induced inflammation. We used the carrageenin-air-pouch method which was developed as an experimental model covering not only acute exudative but also chronic proliferative stages of the inflammatory processes (4).

### MATERIALS AND METHODS

An inflammation in the form of carrageenin-air-pouch was induced on the back of male Donryu rats according to the procedure of Tsurufuji et al. (4). In rats weighing 130–160 g, 7 ml of air was injected s.c. on the back to make an air pouch. After 24 hr, 4.0 ml of 2 (w/v) % solution of Seakem 202 carrageenin (Marine Colloid Inc., Springfield, N.J., U.S.A.) was injected into the air pouch already formed. Radioiodinated human serum albumin ( $^{131}\text{I}$ -HSA, Dainabot Co., Tokyo, 5  $\mu\text{Ci}/\text{mg}$ ) was used after purification by gel filtration as an indicator for the measurement of vascular permeability (5).  $^{131}\text{I}$ -HSA (4  $\mu\text{Ci}/\text{kg}$  in 1 ml of 0.9% NaCl) was given i.v. 4 hr after the last injection of progesterone,

and 30 min later the rats were sacrificed by cutting carotid artery. The entire exudate and the pouch of the granulation tissue were harvested. The exudate was centrifuged at  $2,000 \times g$  for 10 min at  $4^\circ\text{C}$ . The radioactivity of the supernatant (1 ml) was measured in a well type scintillation spectrometer as described in a previous paper (5). Total radioactivity in the entire pouch fluid of each rat was calculated and expressed in terms of the percentage of the radioactivity injected and used as an index of the vascular permeability of the inflamed tissue.

Collagen of the granulation tissue was extracted as gelatin by autoclaving the tissue with distilled water. The extraction was done twice and the resulting collagen-free residue was referred to as the noncollagen protein fraction. The amounts and the radioactivities of collagen and noncollagen protein fractions were measured according to the procedure described previously (6). Briefly, collagen extracted as gelatin was hydrolyzed with 6 N HCl at  $105^\circ\text{C}$  for 16 hr. The amount of hydroxyproline in the hydrolysate was measured by the method of Kivirikko et al. (7). The radioactivity of hydroxyproline in the hydrolysate was measured after converting hydroxyproline into pyrrole which was purified by passing through a silicic acid column. The purified pyrrole solution (15 ml) was mixed with a liquid scintillator (2 ml of 1.5% 2,5-diphenyloxazole in toluene) and counted. Noncollagen protein was homogenized in distilled water and a homogenous suspension of noncollagen protein was made by vigorous shaking. The amount of noncollagen protein in an aliquot of the suspension was measured by the method of Lowry et al. (8). Another aliquot of the suspension was solubilized with the aid of 1 ml of Soluene 100 and then 16 ml of a liquid scintillator (0.6% 2,5-diphenyloxazole in toluene) was added. The radioactivity was measured in a Packard liquid scintillation spectrometer (model 3202) equipped with automatic external standardization.

## RESULTS

*Effect of progesterone on the early phase of the inflammation:* In the early phase of the inflammation (day 0–day 4), effect of progesterone on the vascular permeability and on the formation of granulation tissue was studied. Progesterone (pregn-4-ene-3,20-dione; Merck),

TABLE 1. Effect of progesterone on the early phase of the carrageenin-induced inflammation

	Control	Progesterone
No. of rats	9	8
Net body wt. (g)	$176 \pm 3$	$175 \pm 4$
Granulation tissue, wet wt. (g)	$2.93 \pm 0.17$	$1.68 \pm 0.23^{**}$
Exudate (g)	$6.05 \pm 0.62$	$4.09 \pm 0.70^*$
Vascular permeability	$0.39 \pm 0.04$	$0.22 \pm 0.03^{**}$
Collagen hydroxyproline (mg) in whole tissue	$2.45 \pm 0.21$	$1.24 \pm 0.12^{**}$
Collagen hydroxyproline (mg)/g wet wt.	$0.84 \pm 0.07$	$0.77 \pm 0.05$
Noncollagen protein (mg) in whole tissue	$71.8 \pm 4.54$	$43.7 \pm 4.70^{**}$
Noncollagen protein (mg)/g wet wt.	$24.5 \pm 0.85$	$27.0 \pm 2.02$

Data are shown as mean  $\pm$  S.E. Statistically significant difference of the mean against control is indicated by \* ( $P < 0.05$ ) and \*\* ( $P < 0.01$ ).

in a dose of 1 mg dissolved in 1 ml sesame oil per kg body weight, was injected every 12 hr into the carrageenin-air-pouch for 4 days commencing on the day of the carrageenin injection. Control animals were given the vehicle only (1 ml sesame oil/kg body wt.). The early phase of the carrageenin-induced inflammation was suppressed by the progesterone treatment. Wet weight of the granulation tissue, exudate volume in the pouch and the local vascular permeability were significantly inhibited (Table 1). Concomitantly, collagen content and noncollagen protein content in the entire granulation tissue from the progesterone group were significantly lower than in the control group.

*Effect of progesterone on the chronic phase of the inflammation:* Effect of progesterone on the chronic phase of carrageenin-induced inflammation was studied in comparison with the effect of a potent anti-inflammatory steroid, hydrocortisone acetate. On day 6 after carrageenin injection, granuloma-bearing rats were injected with 50  $\mu$ Ci/kg of L-[ $^3$ H]-proline (generally labeled, 63 Ci/mmol) into the granuloma pouch and the rats were separated into five groups (7 rats/group). The first group was sacrificed 24 hr later and served as the 7-day control. The second, the third and the fourth groups were injected respectively with progesterone (1 mg/kg in 1 ml sesame oil) or with hydrocortisone acetate (0.5 mg/kg in the low dose group and 1 mg/kg in the high dose group, in the form of a suspension in 0.5% carboxymethylcellulose aqueous solution, Nippon Merck-Banyu Co., Ltd., Tokyo) into the granuloma pouch every 12 hr over the period from day 7 to day 11. The fifth group was injected with the vehicle (sesame oil) only. In addition, 5  $\mu$ Ci/kg of L-[U- $^{14}$ C]proline (274 mCi/mmol) was injected i.p. 4 hr after the last injection of the drugs or the vehicle and 16 hr later (on day 12) the rats were killed.

The results are summarized in Table 2. The progesterone treatment (1 mg/kg) produced a significant decrease both in the wet weight of the granulation tissue and in the amount of the exudate. This inhibitory effect of progesterone was enhanced somewhat by the treatment of an increased dose (2 mg/kg) of progesterone. The effect of progesterone on the vascular permeability in the chronic phase was also examined in a separate experiment, in which progesterone (1 mg/kg) was repeatedly injected as described above, and each rat was injected i.v. with 4.3  $\mu$ Ci of purified  $^{131}$ I-HSA/kg 4 hr after the last injection of progesterone, and 30 min later the exudate was harvested. The vascular permeability in the inflamed tissue was suppressed by progesterone by 50%; the value for the control group was  $0.31 \pm 0.05\%$  and for progesterone-treated group  $0.15 \pm 0.01\%$  (mean  $\pm$  S.E. of 7 rats).

With respect to degradation of collagen, more than half of [ $^3$ H]-collagen in the granulation tissue disappeared during the period from day 7 to day 12 in both the control and the progesterone-treated groups. There was no significant difference in the collagen degradation between these two groups. On the contrary, hydrocortisone acetate significantly inhibited collagen breakdown (Table 2). On the other hand, the total amounts of [ $^3$ H]-noncollagen protein of the granulation tissue in the progesterone and hydrocortisone acetate groups were significantly lower than that of 12-day control, though there was no difference between both the values of the progesterone and hydrocortisone acetate groups (Table 2). These results suggested that progesterone and hydrocortisone acetate enhanced the de-

TABLE 2. Effect of progesterone and hydrocortisone acetate on the chronic phase of carrageenin-induced inflammation<sup>a)</sup>

	7-day control	12-day control	Progesterone 1.0 mg/kg	Hydrocortisone	
				0.5 mg/kg	1.0 mg/kg
Net body wt. (g)	175 ± 5	193 ± 6	199 ± 3	189 ± 4	196 ± 5
Granulation tissue, wet wt. (g)	5.65 ± 0.20	4.88 ± 0.16	3.94 ± 0.27**	3.97 ± 0.38***	2.72 ± 0.09*
Exudate (g)	25.3 ± 1.3	38.3 ± 0.9	26.8 ± 2.2*	15.6 ± 3.3*	9.2 ± 2.2*
Collagen					
collagen hydroxyproline (mg) in whole tissue.	9.25 ± 0.52	10.75 ± 0.39	8.92 ± 0.96	10.97 ± 1.06	8.48 ± 0.32*
collagen hydroxyproline (mg)/g wet wt.	1.66 ± 0.12	2.20 ± 0.04	2.23 ± 0.10	2.79 ± 0.14*	3.13 ± 0.12*
radioactivity of <sup>3</sup> H-collagen hydroxyproline in whole tissue. (dpm × 10 <sup>-4</sup> )	13.2 ± 0.88	5.86 ± 0.23	5.61 ± 0.38	8.88 ± 0.58*	8.29 ± 0.39*
incorporation of <sup>14</sup> C-proline into collagen; radioactivity of <sup>14</sup> C-collagen hydroxyproline in whole tissue. (dpm × 10 <sup>-3</sup> )		1.90 ± 0.14	1.43 ± 0.19	0.32 ± 0.12*	0.35 ± 0.05*
Noncollagen protein					
protein (mg) in whole tissue.	213 ± 12.5	229 ± 9.4	183 ± 16.9***	119 ± 7.7*	93 ± 2.8*
protein (mg)/g wet wt.	37.7 ± 1.60	46.9 ± 0.71	46.2 ± 1.21	31.2 ± 2.47*	34.2 ± 1.29*
radioactivity of <sup>3</sup> H-noncollagen protein in whole tissue. (dpm × 10 <sup>-3</sup> )	7.74 ± 0.53	2.24 ± 0.12	1.62 ± 0.10*	1.60 ± 0.05*	1.52 ± 0.08*
incorporation of <sup>14</sup> C-proline into noncollagen protein; radioactivity of <sup>14</sup> C-noncollagen protein in whole tissue. (dpm × 10 <sup>-3</sup> )		12.3 ± 0.77	10.0 ± 1.16	6.88 ± 0.75*	3.50 ± 0.26*

a) Each group included 7 rats. All data are the mean ± S.E. Statistically significant difference of the mean against 12-day control is indicated by

\*(P &lt; 0.01), \*\*\*(P &lt; 0.02) and \*\*\*(P &lt; 0.05).

gradation of noncollagen protein in the granulation tissue.

The effect of progesterone and hydrocortisone acetate on the incorporation of [ $^{14}\text{C}$ ] proline into collagen and noncollagen protein was also studied. The results are shown in Table 2. It is unlikely that progesterone exerted any effect on the syntheses of collagen and noncollagen protein, since the incorporation of [ $^{14}\text{C}$ ]proline into collagen and noncollagen protein was not apparently affected by progesterone. On the contrary, hydrocortisone acetate appeared to inhibit the incorporation of [ $^{14}\text{C}$ ]proline into both the proteins.

## DISCUSSION

The present experiments clearly demonstrated that progesterone inhibited vascular permeability response and development of granulation tissue in the early phase of carrageenin-induced inflammation in rats. Lindhe and Sonesson (2) also found that progesterone reduced the thickness of the granuloma pouch wall induced by croton oil in rats. Nyman et al. (9), however, demonstrated that progesterone had no effect on the induction of granulation tissue when steel cylinders were implanted in oophorectomized rabbits. The discrepancy between our results and those of Nyman et al. might be accounted for by difference in the dose of progesterone, in the animal used and in the experimental model of inflammation. In the chronic, proliferative phase as well as in the early phase of the inflammation, progesterone exerted anti-inflammatory effects not only in suppressing accumulation of exudation fluid but in alleviating proliferative response as reflected in the wet weight of the granulation tissue. Aspirin, indomethacin and phenylbutazone were shown to be ineffective in the chronic, proliferative phase of the carrageenin-induced inflammation, though such compounds were effective in the early phase (10). In these respects, progesterone exerts a broader range of anti-inflammatory activity.

Influence of progesterone on collagen and noncollagen protein metabolism in the granulation tissue appears to be somewhat different from that of glucocorticoids. Hydrocortisone acetate inhibited collagen breakdown in the chronic phase of inflammation (Table 2). This observation is consistent with our findings reported previously in relation to beta-methasone disodium phosphate (11). Koob et al. (12) demonstrated that hydrocortisone ( $10^{-7}$  M) and dexamethasone ( $10^{-8}$  M) inhibited the appearance of collagenase in cultures of normal human skin, human rheumatoid synovium and rat uterus. Therefore, inhibition of collagen breakdown in the hydrocortisone acetate group may have been caused by a suppression of collagenase production in the granulation tissue. Jeffrey et al. (13) reported that progesterone added in physiologic concentrations to tissue cultures of rat postpartum uterus almost completely blocked collagen breakdown and appearance of collagenolytic enzyme in the medium. In the granulation tissue, however, collagen breakdown was not affected by progesterone (Table 2). It is quite reasonable that progesterone protects postpartum uterine tissue collagen from the catabolic process, since the physiological role of this hormone is to maintain hypertrophic uterine tissue during pregnancy. The total amount of [ $^3\text{H}$ ]noncollagen protein in the granulation tissue of hydrocortisone group was significantly lower than that of 12-day control group, suggesting that hydrocortisone acetate enhanced

the degradation of noncollagen protein. However, there is the alternative possibility that re-utilization of free [ $^3\text{H}$ ]proline liberated with degradation of [ $^3\text{H}$ ]proline-containing protein in the body was inhibited by hydrocortisone, since incorporation of [ $^{14}\text{C}$ ]proline into noncollagen protein in the granulation tissue was shown to be markedly inhibited by hydrocortisone (Table 2). In the case of progesterone, however, it is evident that this hormone enhanced the involution of pre-existing granulation tissue by means of an increased degradation of noncollagen protein without affecting the synthetic ability for noncollagen protein. Such a concept is agreeable with the finding that significant nitrogen loss occurs during treatment with progesterone of normal human subjects (14, 15).

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