

Rebound of Vascular Permeability Response and Its Inhibition by Hydroxyurea in Granulomatous Inflammation Following Withdrawal of Glucocorticoid Therapy

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Glucocorticoid therapy induces rapid involution of chronic granulomatous inflammation which has been provoked by injecting carrageenin solution subcutaneously in rats. Cortisol acetate injected into the granuloma pouch at a dose of 3 mg/kg/day for 3 days caused a marked involution of the granulomatous inflammation. After withdrawal of the steroid treatments, however, rebound of the inflammation took place resulting in a rapid recovery not only in the wet weight and the dry weight of the granuloma but also in vascular permeability response and the volume of the exudate.

Determination of vascular permeability of the granulomatous tissue were performed with the aid of radioiodinated human serum albumin which was injected intravenously 30 min before sacrifice. Vascular permeability was expressed in terms of radioactivity exuding into the granuloma pouch fluid for the 30 min. By repeated administrations at 12 hr intervals with hydroxyurea (250 mg/kg \times 6, injected into the granuloma pouch) after the withdrawal of the steroid treatments, rebound of the inflammation not only in proliferative phases but also in exudative phases was suppressed almost completely.

Glucocorticoids are widely used in the clinical medicine as potent anti-inflammatory agents, though reactivations of the diseases are often encountered following the cessation of the steroid therapy.²⁾

In a previous paper we described a proper animal model of the reactivation (rebound phenomenon) which took place in a granulomatous inflammation provoked by carrageenin in rats.³⁾ This animal model of a chronic granulomatous inflammation called carrageenin granuloma pouch method established by Fukuhara and Tsurufuji⁵⁾ has been intensively used to investigate on the proliferative phase of the inflammation.⁶⁻¹¹⁾ Analysis of biochemical effects of the glucocorticoids on this inflammatory tissues suggested that interference with desoxyribonucleic acid (DNA) synthesis exerted a certain role in the anti-inflammatory actions of the steroids.⁴⁾

In the course of the rebound phenomena following the withdrawal of glucocorticoid therapy DNA was shown to be reactivated in the early phase.³⁾ The purpose of the present experimental work was to investigate whether recurrence of cell proliferation, as shown by the enhancement of DNA synthesis in the course of the rebound of the granulomatous inflam-

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mation, correlates or not with the rebound of vascular permeability response. Hydroxyurea, a specific inhibitor of DNA synthesis,¹²⁻¹⁴⁾ was introduced in order to block the reactivation of DNA synthesis of granulomatous tissues. Experimental results showed that hydroxyurea interfered not only with the reactivation of granuloma proliferation but also with the rebound of vascular permeability responses.

Materials and Methods

Induction of Carrageenin Granuloma Pouch—Young male rats of the Donryu strain, aged 45–48 days, weighing 130–150 g were used. All animals received laboratory rat chow (Funabashi Farm Inc., Chiba) and tap water throughout the experiment. According to the methods described in a previous report⁵⁾ a granuloma pouch was induced by injecting 2% solution of carrageenin (Seakem 202, Marine Colloid Inc., Springfield, N.J., U.S.A.) in 0.9% NaCl into the air sac which had been produced on the preceding day on the dorsum of rats. The day of carrageenin injection was designated as day 0. On the day of the first injection of hydrocortisone acetate, the animals were so grouped as to make uniform grouping with respect to the distribution in the size of the granuloma pouch and the body weight. Animals bearing granuloma of solid mass were not used.

Drug Treatments—The suspension of the fine powder of cortisol acetate in the daily dose of 3 mg/kg was directly injected into the granuloma pouch during the days 5–7. The dose and the term of the dosing were selected according to the results of a previous paper³⁾ in order to give an appropriate condition for observing the rebound of the granuloma. The suspension was prepared by using 0.5% carboxymethylcellulose (CMC) aqueous solution as a vehicle in a Vir-Tis 45 homogenizer operating 2 min at a maximum speed. Control animals were given the vehicle only.

In the case of rats treated with hydroxyurea, 6 repeated injections of the drug in the granuloma pouch at a single dose of 250 mg/kg in 0.2 ml of pyrogen-free sterile 0.9% NaCl were given at 12 hr intervals. The 1st injection of hydroxyurea was performed 12 hr after the last injection of cortisol acetate. During the period of hydroxyurea treatments a procedure of pair-feeding was performed in order to avoid the irregularity in body weight gain among groups since growing rate of hydroxyurea group declined slightly.

Measurement of Vascular Permeability—In order to measure vascular permeability of the granuloma pouch, animals were injected *i.v.* with 1 μ Ci of ¹³¹I-labeled human serum albumin (¹³¹I-HSA) solution in 0.2 ml of 0.9% NaCl under light ether anesthesia. The animals were killed 30 min later by cutting the carotid artery. The pouch wall of the granuloma was incised to open and the exudate in the pouch was harvested in a polyethylene beaker. The gross amount of the exudate was measured by its weighing assuming its specific of gravity to be 1.0. The exudate thus obtained was centrifuged at 2500 rpm for 15 min at 1° to remove clusters dead cells aggregating together with fibrous debris. One ml aliquot of the exudate supernatant was transferred into a plastic test tube for counting radioactivity in a scintillation counter (Aloka Autowell Gamma System, JDC-751). Vascular permeability of the granuloma pouch was expressed in terms of radioactivity which exuded into the pouch exudate. The radioactivity of the exudate was expressed as percentage of the total amount of injected ¹³¹I-HSA.

Dry Weight of the Granuloma—Granuloma tissue was harvested and the wet weight of the granuloma was weighed. An aliquot of the tissue was dried in a vacuum drying oven at 100° to obtain the constant dry weight.

Results

Rebound of Granuloma Tissue after the Withdrawal of Glucocorticoid Treatment

As shown in Table I, hydrocortisone acetate treatment at a dose of 3 mg/kg/day for 3 days caused a marked involution of the granuloma tissue. When assayed on day 8 (1 day after the last injection of the steroid) the wet weight decreased markedly (44.7% inhibition). On day 9 (2 days after the last injection of the steroid), rebound in the wet weight was not yet seen at all. On day 10 (3 days after the last injection), however, sudden increase in the wet weight of the granuloma occurred, indicating complete recovery in the wet weight. These results are virtually identical with those of a previous study.³⁾ The dry weight of the granuloma

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showed the same pattern of changes as the wet weight. A marked decrease (40% inhibition) was given on day 8, and almost complete recovery was attained 2 days later.

TABLE I. Rebound of Inflammation as Reflected in the Wet Weight and Dry Weight of the Granuloma

Treatment and granuloma age	No. of rats	Granuloma wet wt. (g)	Granuloma dry wt. (g)
Control, day 8	7	4.77±0.36	0.735±0.084
Cortisol acetate (3 mg/kg/day×3), day 8	7	2.64±0.13 (55.3%) $p<0.001$	0.436±0.017 (59.3%) $p<0.01$
Cortisol acetate (3 mg/kg/day×3), day 9	5	2.98±0.28	0.458±0.042
Control, day 10	7	4.32±0.35	0.731±0.056
Cortisol acetate (3 mg/kg/day×3), day 10	7	4.74±0.49	0.699±0.059

Data are shown as mean ± S.E. Figures in the parenthesis represent the percentage of Control. Cortisol acetate was injected into the pouch on days 5, 6, and 7, and then granuloma was harvested on days 8, 9, and 10.

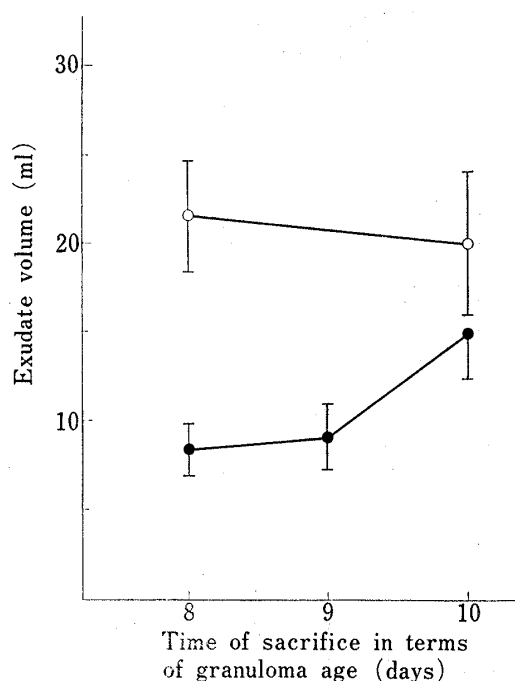


Fig. 1. Rebound of the Granulomatous Inflammation as reflected in the Volume of the Exudate

The last injection of hydrocortisone acetate into the granuloma pouch was given on day 7. Each point is the mean of 7 animals except the value of the day 9 in which 5 animals were used.

(—○—: control, —●—: treated) A vertical line at each point represents the S.E. of the mean.

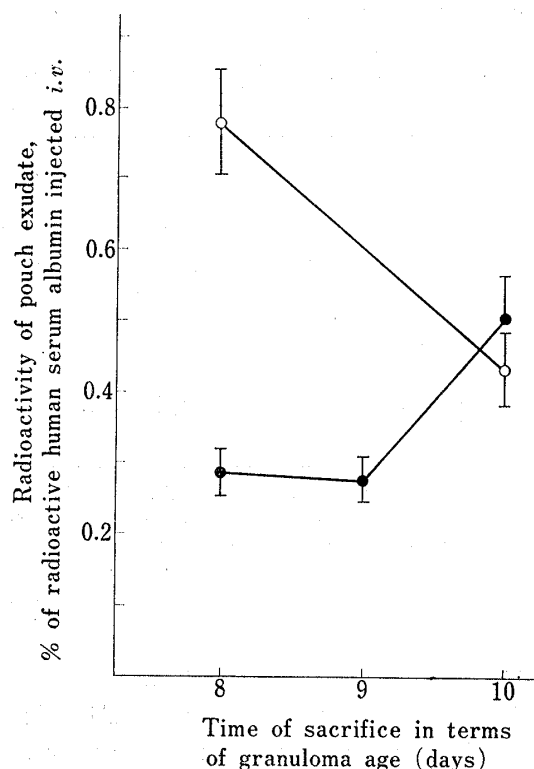


Fig. 2. Rebound of the Vascular Permeability Response in Granuloma Pouch, Following Cessation of Hydrocortisone Acetate Injections

Radioiodinated human serum albumin was injected i.v. 30 min before harvesting the pouch exudate.

Each point is the mean of 7 animals except the value of the day 9 in which 5 animals were used. A vertical line at each point represents the S.E. of (—○—: control, —●—: treated) the mean.

Rebound of Vascular Permeability Response Following the Cessation of Glucocorticoid Treatments

Exudate volume in the granuloma pouch was decreased markedly by the steroid treatment (60.7% inhibition) as indicated in the data of day 8 in Fig. 1. Substantial rebound in the exudate volume was not seen on day 9, but on day 10 the volume was increased to some extent. The rebound in the exudative reaction could be detected more effectively by direct measurements of vascular permeability in the granuloma pouch with the aid of radioactive human serum albumin, as shown in Fig. 2. The amount of radioactivity exuding into the pouch fluid of the granuloma was markedly depressed (63.3% inhibition) in the steroid-treated rats of day 8 groups. Two days later (on day 10) apparent value of the vascular permeability in the steroid-treated group surpassed control level (117% of control), indicating the reactivation of vascular permeability response.

Inhibitory Effect of Hydroxyurea on the Rebound

A series of experiments were performed for investigating the effects of hydroxyurea on the rebound of the granulomatous inflammation which occurred after withdrawal of the steroid treatment. Almost complete recovery in the granuloma wet weight in the steroid-treated animals sacrificed on the day 10 (3 days after withdrawal of the steroid treatment) was again confirmed in this series of experiments as shown in Table II. Assaying of vascular permeability in this group of rats also reconfirmed the rebound in exudative response. On the other hand, when repeated injections with hydroxyurea followed the cessation of the steroid treatments, rebound of the inflammation was markedly inhibited. Wet weight of the granuloma in the hydroxyurea-group remained 61.5% of the control group and the vascular permeability remained 55.4%.

TABLE II. Inhibition by Hydroxyurea of the Rebound as Observed in the Wet Weight and Vascular Permeability of the Granuloma

Groups	No. of rats	Granuloma wet wt. (g)	Radioactivity of exudate (% of dose)
Pair-fed-control, day 10	6	4.99±0.36	0.74±0.05
Cortisol acetate (3mg/kg/day×3) and rebound, day 10	5	4.34±0.43	0.72±0.18
Cortisol acetate (3mg/kg/day×3) +	5	3.07±0.33	0.41±0.08
Hydroxyurea (250 mg/kg/day×6) day, 10		(61.5%) $p<0.01$	(55.4%) $p<0.01$

Data are shown as mean ± S.E. Figures in the parenthesis represent the percentage of the control group. Cortisol acetate was injected into the pouch on days 5, 6, and 7, and then hydroxyurea injections into the pouch were performed at intervals of 12 hr.

Discussion

It has been demonstrated that carrageenin granuloma pouch method is a typical model of chronic inflammatory response.⁵⁾ This inflammation begins with cellular infiltration provoked by the injection of carrageenin, followed by proliferation of fibroblasts to make a granuloma pouch in which inflammatory exudate fluid appears. The amount of the pouch fluid increases gradually during the period of days 5—9 up to around 20 ml and then levels off.

Administration of glucocorticoid induces marked involution of the preexisting granulomatous inflammation resulting in rapid declines in both the proliferative and exudative phases of the inflammatory responses. Although glucocorticoids are so effective as to alleviate a number of clinical and experimental inflammatory diseases, many untoward side effects bring about profound troubles. Rebound of disease symptoms after the withdrawal of gluco-

corticoid treatments is one of the troubles often encountered in clinical medicine. We have been doing laboratory works to investigate biochemical aspects of rebound phenomena using carrageenin granuloma pouch method in an attempt to find possible methods to suppress the rebound symptoms. Some of the results were reported previously.^{3,4)} Hydroxyurea was selected in a previous work as an agent to depress DNA synthesis of the granuloma under the rebound phase.⁴⁾ In the present experiment it was clearly shown that repeated administration of hydroxyurea after withdrawal of glucocorticoid treatments could alleviate the rebound of vascular permeability response. The present doses of hydroxyurea, 6 repeated injections of each 250 mg/kg at 12 hr intervals, was shown in the previous paper to be enough for suppressing DNA synthesis of the granulomatous tissues. Although exact correlation of the reactivation of DNA synthesis with the rebound of vascular permeability response is not clear yet, possible suppression of capillary vessel proliferation through blocking DNA synthesis may be responsible for the suppression of the rebound in vascular permeability response.