Antinephritic Effect of Prostaglandin E₁ on Serum Sickness Nephritis in Rats (4)
Enhanced Clearance of Macromolecules by the Reticulo-Endothelial System with Prostaglandin E₁

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Abstract—Function of the reticulo-endothelial system (RES) was determined by the carbon clearance technique in rats. Rats in the previous phase of nephritis showed reduced function of the RES with circulating immune complexes compared with normal rats. Prostaglandin E₁ (PGE₁) at 1.0 mg/kg, s.c., resulted in significant recovery in the impaired RES activity of the rats in the previous state of nephritis. Dipyridamole, 400 mg/kg, p.o., slightly enhanced function of the RES, but 20 mg/kg azathioprine, p.o., did not. Thus, the enhanced RES activity with PGE₁ can explain part of the antinephritic effect of PGE₁.

There is much evidence indicating that the disposal of circulating immune complexes is accomplished substantially by the cells of the reticulo-endothelial system (RES) (1, 2). Dysfunction of the RES could be responsible for deposition of immune complexes in the glomeruli (3, 4). Additionally, glomerular immune deposits trigger the development of glomerulonephritis (5, 6), although many factors are implicated in the process. We have previously demonstrated that prostaglandin E₁ (PGE₁) attenuates the progress of serum sickness nephritis (immune complex nephritis) in rats (7, 8). Therefore, in the present study, we attempted to elucidate whether PGE₁ could enhance the clearance of circulating macromolecules by the RES.

The drugs used in the experiments were PGE₁ (Funakosi Yakuhin), dipyridamole (Dip., Boehringer Ingelheim, Ltd.) and azathioprine (Aza., Tanabe Seiyaku). PGE₁ was dissolved in absolute ethanol and diluted with phosphate-buffered saline as reported previously (9), and given s.c. at 0.1 and 1.0 mg/kg, Aza. at 20 mg/kg and Dip. at 200 and 400 mg/kg were suspended in 5% gum arabic solution, and then orally administered.

Five groups of 7 to 10 animals were used in this study. Serum sickness nephritis was induced in male SD rats (Chubu Keari) weighing about 180 g by immunization with rabbit serum albumin (RSA, Sigma) (twice at a 4-week interval) and also by daily i.v. injection (started 2 weeks after the first immunization) of RSA over a period of 3 weeks as previously reported (9).

At 5 weeks after the first immunization with RSA, the rats received once each agent prior to being tested by the carbon clearance technique to determine the function of the RES according to the method of Halpern et al. (10).

In the present carbon clearance technique, India ink (Pilot Co., Ltd.) containing 60 mg/ml of carbon particles was diluted with saline containing 1% gelatin to prepare a solution of 32 mg/ml India ink. This solution was placed at 37°C overnight and injected i.v. at a dose of 16 mg/100 g body weight. A blood sample of exactly 25 μl was obtained by puncturing the retro-orbital venous with a capillary glass pipette before the injection of carbon solution (0) and 10, 20, 50, 100 min after the injection of carbon. Each blood sample was gently placed in a test tube.
containing 2 ml of 0.1% Na\textsubscript{2}CO\textsubscript{3} to ensure hemolysis, and the amount of carbon in the blood was determined electrophotometrically at 660 nm with a standard curve.

The K value, granuloplectic index for the RES, was calculated from the clearance curve obtained according to the mathematical formula proposed by Brozzi et al. (11) and represents the functional activity of the RES.

\[ C = C_0 10^{-Kt} \quad K = \log \frac{C_0}{C} \]

C is the concentration of carbon in the blood at the time T, and C\textsubscript{0} is the blood concentration of carbon just after the injection.

The results are presented as the mean±S.D. and were statistically analyzed by the F-test, unpaired t-test and Mann-Whitney U-test. Differences at the level of P<0.05 were considered significant.

As shown in Table 1, normal rats had a K value of 5.99×10\textsuperscript{-3}, whereas the injection of RSA significantly reduced the K value to 4.01×10\textsuperscript{-3}. The K value represents the ability of the RES-cells to ingest carbon particles, so the K value indicates the functional activity of the RES (11). The current study was performed 5 weeks after the first immunization with RSA. On the other hand, in the previous report (12), we demonstrated that abnormal urinary protein was detected in the rat injected with BSA at 7 weeks after the first immunization, and immune complexes were found in the glomerulus at 5 weeks after the first immunization. Thereafter, serum sickness nephritis became more severe by the injection of RSA with advancing time. Therefore, this result shows that the immune complexes which are induced by the repeated injection of RSA cause the failure of RES function before the development of serum sickness nephritis.

Additionally, this suggests the following events in the rats treated with RSA: 1) the RES began to be saturated with circulating RSA-anti-RSA immune complexes at around 5 weeks after the first immunization. 2) The immune complexes may be kept in the circulation without ingestion by RES cells. 3) The immune complexes gradually deposit in the kidney, and 4) Serum sickness nephritis is induced by the deposited immune complexes. The present results are supported by those of Haakenstad and his colleagues (2, 3) who used \textsuperscript{125}I-soluble immune complexes consisting of human serum albumin and rabbit anti-human serum albumin in mice and that of Hoffsten et al. (13) who evaluated the function of the RES in normal mice and in nephritic mice induced by lymphocytic choriomeningitis virus infection using heat-aggregated human immunoglobulin and colloidal carbon.

As can be seen in Table 1, 124.2% enhancement of the K value was observed in the group given 1.0 mg/kg PGE\textsubscript{1}, suggesting that PGE\textsubscript{1} can resume the functional activity of the RES. Although we have not measured the anti-RSA antibody titer after the administration of PGE\textsubscript{1}, we demonstrated that consecutive administration of PGE\textsubscript{1} slightly suppressed the anti-RSA antibody titer (9).

### Table 1. Effects of drugs on functional activity of reticulo-endothelial system

| Groups | N  | Dose  | Route | K value\(\times10^{-3}\) |
|--------|----|-------|-------|--------------------------|
| Normal | 10 |       |       | 5.99±1.51                |
| RSA\textsuperscript{b} | 8  |       |       | 4.01±1.25\*              |
| PGE\textsubscript{1} | 8  | 0.1   | s.c.  | 4.19±1.85                |
|       | 8  | 1.0   | s.c.  | 6.47±1.94*               |
| Dip.  | 7  | 200   | p.o.  | 5.51±1.96                |
|       | 7  | 400   | p.o.  | 4.79±2.32                |
| Aza.  | 8  | 20    | p.o.  | 3.86±0.72                |

Results are the mean±S.D. Significant difference from the normal: \*P<0.01 and the control: \*P<0.01.

\textsuperscript{a}: granuloplectic index \textsuperscript{b}: rats had been i.v. injected with RSA over 3 weeks. Drugs were administered once on the next day after the last injection of RSA. Dip.: dipyridamole. Aza.: azathioprine.
We previously thought that the suppressive effect of PGE₁ on antibody titer could be ascribed to the inhibitory effect of PGE₁ on the production of antibody (9). However, in addition to the present finding, the suppressive effect of PGE₁ on antibody titer was less than that of azathioprine (9). Therefore, we now consider that suppressed antibody titer with PGE₁ may be attributed to the enhanced clearance of immune complexes by the RES with PGE₁, and the previous finding (9) supports the present result.

This effect of PGE₁ may be due to the strong inhibitory effect of PGE₁ on platelet aggregation (14) and augmenting effect on blood flow. We could demonstrate that Dip. also increased, although not significantly, the functional activity of the RES, but azathioprine did not. We determined the inhibitory effects of PGE₁ and Dip. in vitro on collagen-induced rat platelet aggregation (15). PGE₁ was 1,000 times stronger in inhibiting platelet aggregation in Dip. It is likely that this inhibitory effect on platelets may be reflected in the present results. That is, the RES-cells have to encounter the circulating immune complexes in order to exclude them from the circulation. If the RES ingest a too large amount of immune complexes, immune complexes in the RES could stimulate platelets to aggregate in the microcirculation of the RES itself because immune complexes can induce platelet aggregation (16, 17). Consequently, blood flow into the RES may be decreased, and then the chance for the RES-cells to encounter the immune complexes may be diminished. This situation leads to the sustaining of immune complexes in the circulation and to their deposition in the glomeruli. In the nephritic animals, PGE₁ could very markedly inhibit the platelet aggregation in the microcirculation; and furthermore, the vasodilating action of PGE₁ could recover the blood flow in the microcirculation. Through these actions, the RES can ingest circulating immune complexes again.

There is another possibility to explain the present results. Nishi et al. (1) provided evidence that the circulating immune complexes can induce the loss of Fc and C3 receptor function of Kupffer cells, the RES-cells, in vivo, suggesting that this can result in diminished clearance of circulating immune complexes. Therefore, PGE₁ may restore the impaired Fc and C3 receptor function of Kupffer cells through the direct effect on these receptors. This remains to be clarified in order to fully understand the mechanism of PGE₁ on the antinephritic effect.

In conclusion, the present result, i.e., increasing the clearance with PGE₁, may explain part of the antinephritic effect of PGE₁ on nephritis induced with immune complexes.

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