Effects of Prostaglandin E₁ and Dipyridamole on Disposal of Colloidal Carbon via Glomerular Mesangial Channels

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Abstract—We investigated the effects of prostaglandin E₁ (PGE₁) and dipyridamole on the disposal of macromolecules via mesangial channels to understand the antinephritic effect of PGE₁. To increase glomerular carbon, rats were injected i.v. first with anti-glomerular basement membrane (GBM) serum and then with 30 mg/100 g body weight of carbon. Carbon in the glomerulus was detected with an image analyzer. Administration of anti-GBM serum resulted in about a 2 to 3-fold increase in glomerular uptake of carbon in the normal rats. To investigate the day-to-day situation of glomerular carbon, the right kidney was removed the day after carbon injection, and the left kidney was isolated on days 7, 14 and 21. By day 21, glomerular carbon had decreased significantly. Rats with carbon received PGE₁ 0.1 x 2 and 1.0 x 2 mg/kg, s.c., and dipyridamole 200 and 400 mg/kg, p.o., for 10 days after the isolation of the right kidney. PGE₁ administration did not cause a decrease in the amount of glomerular carbon compared with that of the control. However, the dipyridamole group showed a more rapid decrease of glomerular carbon. It is concluded that PGE₁ has no effect on the mesangial channels, but dipyridamole can accelerate the disposal of glomerular carbon by way of the mesangial channels.

In previous studies, we observed that prostaglandin E₁ (PGE₁) significantly inhibited an increase in the number of glomerular immune complexes in serum sickness nephritis (1, 2). Additionally, we suggested that PGE₁ enhances the processing and/or the disposal of the glomerular immune complexes in the mesangium. A number of possible disposal routes have been discussed, including movement by way of the glomerular stalk to the juxtaglomerular zone (mesangial channels), degradation by phagocytosis and regurgitation into the glomerular capillary (3). The mesangial channels have been studied by the morphological observation of movement of colloidal carbon from the peripheral mesangium to the glomerular stalk (4). Therefore, we designed an experiment to estimate the effects of PGE₁ and dipyridamole on the disposal of macromolecules by way of the mesangial channels using colloidal carbon as a probe, and to determine with an image analyzer the amount of colloidal carbon taken up to the glomerulus.

Materials and Methods

Male Splague-Dawley rats (Chubu Keari) weighing about 180 g were used in the experiments. Each group consisted of 7 to 10 rats.

To investigate the effect of a subdose (5) of anti-glomerular basement membrane (GBM) serum, rats were injected i.v. with 0.3 ml/rat of anti-GBM serum which did not induce nephritis. The animals then received 30 mg/100 g body weight of colloidal carbon (5, 6). As colloidal carbon, we used India ink (Pilot Co., Ltd.) containing 60 mg/ml of carbon particles, to which 50 units of sodium heparin (Shimizu Seiyaku) was added per 1 ml of ink. The next day, the right kidney was removed, fixed with 10% buffered formalin and embedded in paraffin. In additional experiments, the left kidney
was removed on days 1, 7, 14 and 21 after the isolation of the right kidney to investigate the day course of the glomerular carbon. The isolated kidney was processed as described above.

Furthermore, unilateral kidney-rats were s.c. given 0.1 and 1.0 mg/kg of prostaglandin E₁ (PGE₁) (Funakoshi Yakuhin) twice a day, and 200 and 400 mg/kg of dipyridamole (Boehringer Ingelheim), p.o., for 10 days after the isolation of the right kidney. The control rats were p.o. given 5% gum arabic solution. (Fig. 1)

Sections with the same thickness which were adequate in size for analysis were selected with a light microscope. Carbon in the glomerular cross section (GCS) [specifically, total carbon area (μm²/GCS) and the number of carbon particles (count/GCS)] was determined with an image analyzer (Luzex 500) using 30 glomeruli per one section.

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 levels of P<0.05 were considered to be significant.

Results

1. Effect of subdose anti-GBM serum on glomerular uptake of colloidal carbon (Table 1): In the normal group, total carbon area was 46.7 μm²/GCS, and the number of carbon particles was 15.7/GCS. On the other hand, the group with subdose anti-GBM serum showed about a 3-fold increase in the total carbon area and about a 2-fold increase in the number of carbon particles compared with these in the normal group.

2. Changes in glomerular carbon in unilateral kidney-rat (Fig. 2 and Photo 1): In the unilateral kidney-rat, total carbon area was 303.7 μm²/GCS 1 day after the injection of colloidal carbon. On days 7 and 14, total carbon area had decreased by 50 to 60% as compared to that on day 1, and it decreased to 91.3 μm²/GCS by day 21. The number of carbon particles, however, stayed at a level similar to that of day 1 until day 14. Then there was a significant decrease of 40% in the number of carbon particles by day 21.

3. Effects of drugs on disposal of glomerular carbon (Fig. 3): In the control of PGE₁, total carbon area showed a 54.7% reduction and the number of carbon particles showed a 22.4% reduction compared with day 1. PGE₁ had no effect on total carbon area or the number of carbon particles.

In the control of dipyridamole, total carbon area revealed a 49.0% reduction and the number of carbon particles showed a 2.0% reduction. On the other hand, 200 mg/kg dipyridamole caused a 56.1% decrease in total carbon area and a 25.1% decrease in the

| Table 1. Effect of subdose anti-GBM serum on deposition of carbon particles in glomerulus |
|---------------------------------|-----------------|-----------------|-----------------|
| Subdose of anti-GBM serum       | Animal number   | Carbon particles |
| 0.3 ml/rat                      |                 |                 |
|                                | 8               | total area      |
|                                |                 | μm²/GCS         |
|                                |                 | 46.7±17.7       |
|                                |                 | 134.3±36.5*     |
|                                | 6               | number count/GCS|
|                                |                 | 15.7±6.8        |
|                                |                 | 32.7±7.3*       |

Kidney was isolated one day after the intravenous injection of carbon particles. Results are the mean ±S.D. Significant difference vs. untreated group with anti-GBM serum: *P<0.05. GCS: glomerular cross section.
number of carbon particles, and 400 mg/kg dipyridamole showed a 64.8% decrease in total carbon area and a 53.5% decrease in the number of carbon particles 10 days after the isolation of the right kidney.

Discussion

In previous studies, we observed a great number of immune complexes in the mesangium in serum sickness nephritis (1, 2, 7). Heymann nephritis, another immune complex-type nephritis, showed granular deposition of immune complexes only along the capillary wall (8). Therefore, it seems that the mesangium briskly takes up macromolecules such as immune complexes by serum sickness nephritis. Additionally, the mesangial function which discharges immune complexes from the glomerulus may be injured (9) in serum sickness nephritis.

In the present studies, we injected subdose anti-GBM serum into rats, because we thought that we could more efficiently evaluate the effects of the drugs on the disposal of macromolecules from the glomerulus if the mesangium takes up a large number of macromolecules. In addition, we wanted to reduce the effect of the treatment to augment the uptake of macromolecules into mesangium. Subdose anti-GBM serum caused an increase by 2 to 3 times in the amount of glomerular carbon in the normal rats. This
result agrees with that obtained by Hoyer et al (5) and Mauer et al. (10, 11) who studied the effect of glomerulonephritis in rats on the glomerular uptake of colloidal carbon. However, we observed neither proteinuria nor histological abnormality with the light microscope. Therefore, we injected i.v. subdose anti-GBM serum into rats in the following experiments.

We have attempted here to determine the amount of glomerular carbon with an image analyzer. By this method, the amount of glomerular carbon is presented as a total carbon area and as the count per glomerular cross section, and we can easily follow the day-to-day situation regarding the disposal
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of the carbon from the glomerulus using these figures. Morphologically, aggregated colloidal carbon was located in the peripheral mesangium on day 1. On day 7, total carbon area, but not the count, had decreased, and carbon was observed in the interstitial space around the glomerulus. On day 14, total carbon area had attained the bottom level, and this seemed to be related to a decrease in the count. On that day, the carbon was located at the axis and capillary pole of the glomerulus, and it spread out into the cortical interstitial space. Although the glomerular route of entry into the interstitial space, the lymphatics or the distal tubular cells has not been established (3), we confirmed that the mesangial carbon moved from the peripheral mesangium to the capillary pole of the glomerulus by way of the so-called mesangial channels (3). This result is supported by the evidence of Elema et al. (4) who observed it using electron microscopy.

Mesangium is the connective tissue cells arising in the stalk and extending to the inner aspect of the glomerular capillary. Mesangium has branching cytoplasmic processes and contains a number of organelles including structures such as myosin filaments, suggesting that the mesangium may consist of modified smooth-muscle cells (3). In addition, myosin has been detected in the mesangium by means of immunofluorescent microscopy (12). Therefore, it is likely that mesangium has the ability to constrict and relax in response to stimulants (13). In the present experiments in which we used drugs, 400 mg/kg dipyridamole accelerated the disposal of glomerular carbon, but not PGE1. One possible reason for this acceleration of glomerular carbon disposal is that this drug has an anti-platelet effect (14, 15) and/or a vasodilating effect (16). This possibility, however, is unlikely, because PGE1, which has an anti-platelet effect and a vasodilating effect like those of dipyridamole, did not accelerate the disposal of the glomerular carbon. Ueki et al. (16) reported that dipyridamole relaxed the small intestine in vivo and in vitro. On the other hand, PGE1 strongly constricts the intestinal tract (17). In conclusion, we believe that dipyridamole dilates the mesangial channels and consequently the glomerular carbon is carried out from the mesangium.

Our results, indicating that PGE1 does not accelerate the disposal of glomerular carbon, do not agree with the previous experiments showing that PGE1 may accelerate the processing of immune complexes in the glomerulus (1, 2). The fact that carbon is an inorganic substance and not digested by the mesangial cell and/or by the mesangial residential cells might explain this discrepancy.

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