D-penicillamine-induced enhancement of the delayed hypersensitivity reaction in guinea-pigs

T. HONMA AND Y. NAKAYAMA

From the Department of Pharmacology, Kawasaki Medical School, Kurashiki 701-01, Japan

SUMMARY Intraperitoneal gelatin sponge implants were used in guinea-pigs to examine the effect of D-penicillamine on the delayed hypersensitivity reaction in vivo. When it was administered daily in a dose of 200 mg/kg for 14 days before sensitisation or for 14 or 30 days before challenge, D-penicillamine increased the number of exudate cells during the onset of the delayed hypersensitivity reaction. About 20% more polymorphonuclear cells accumulated in the sponges within 6–12 hours of implantation than did without D-penicillamine. Moreover, mononuclear cells also increased up to 20%, but this effect was apparent only 24 to 72 hours after sponge implantation and if D-penicillamine had been administered immediately before challenge.

Absorbable gelatin sponges which are commonly used for haemostasis can be particularly useful as cell chamber materials because of their capacity to act as artificial spongy tissues. When a gelatin sponge impregnated with an antigen is surgically implanted intraperitoneally in sensitised animals, the interstices of the gelatin sponges become filled with peritoneal exudate cells. The fact that primed peritoneal exudate cells responding to antigen accumulate densely in the gelatin sponges shows that the stages of a delayed hypersensitivity reaction can be assessed in vivo both objectively and quantitatively by analysing serial changes in the accumulation of the exudate cells and expressing this accumulation quantitatively.

In the present study intraperitoneal implants of gelatin sponge were used in animals to examine the effect of D-penicillamine on the delayed hypersensitivity reaction in vivo. A quantitative analysis was made of the serial changes in exudate cells accumulated within gelatin sponges containing ferritin that were implanted intraperitoneally in ferritin-sensitised animals, and studies were made on the chemotactic responses of the exudate cells to D-penicillamine.

Materials and methods

Female English Hartley guinea-pigs weighing 400–550 g were used. Twenty guinea-pigs were investigated per group and 4 guinea-pigs per subgroup or time point.

The guinea-pigs were sensitised by injecting into the foot pads 800 μg ferritin (cadmium free, Nutritional Biochemicals Co.) emulsified in complete Freund's adjuvant (Difco Laboratory). They were challenged 21 days later by implanting gelatin sponges (1×1×0·5 cm, Yamanouchi Pharmaceuticals Co.) into the peritoneal cavity. The guinea-pigs were killed 6 h, 12 h, 24 h, 48 h, or 72 h later. The gelatin sponges were immediately removed, fixed in 10% neutral formalin, and embedded in paraffin. Serial sections 6 to 8 μm thick were stained with haematoxylin and eosin for morphological examination. Ten sections per sponge were measured, and the number of cells per 0·25 mm² of sections was counted. The mean of the cell counts for 4 individual sponges was given.

Group 1–4. All guinea-pigs received gelatin sponges containing 100 μg ferritin (ferritin sponge). They were given no D-penicillamine (group 1) or D-penicillamine (200 mg/kg, Takeda Chemical Industries Ltd) perorally once per day for 14 days before sensitisation (group 2), for 14 days before challenge (group 3), or for 30 days (group 4).

Group 5–6. All guinea-pigs received gelatin sponges impregnated with phosphate buffered saline (PBS sponge) and were given no D-penicillamine (group 5) or D-penicillamine (200 mg/kg) perorally once per day for 14 days before challenge (group 6).

Group 7. Guinea-pigs which were not sensitised or treated with D-penicillamine but received gelatin sponges with 100 μg ferritin served as controls.
Results

The number of exudate cells in the ferritin sponges of ferritin-sensitised guinea-pigs, counted per 0.25-mm² of sections, reached its peak 24 hours after implantation of the ferritin sponges and decreased thereafter (Table 1). When D-penicillamine was administered before the sensitisation or challenge periods, the number of exudate cells in the ferritin sponges increased, whereas the number of cells in the PBS sponges did not increase. After the material was stained with haematoxylin and eosin, the morphology of the nuclei of the cells in the peritoneal exudate that had accumulated in the implanted gelatin sponges could be used to differentiate polymorphonuclear cells (PMN) from mononuclear cells (MN). The exudate cells that accumulated in the ferritin sponge (total numbers given in Table 1) were thus differentiated and counted separately (Table 2). When D-penicillamine was administered prior to the sensitisation or challenge periods, it resulted in an increase in PMN cells in the ferritin sponges in the ferritin-sensitised guinea-pigs within 6–12 hours of their implantation. However, only when the D-penicillamine was administered prior to challenge did it result in an increase in MN cells within 24 hours of implantation. Moreover, MN cells continued to increase at 48 and 72 hours following implantation, eventually exceeding the PMN cells and dominating the exudate cells in the ferritin sponges in the ferritin-sensitised guinea-pigs.

Table 1 Effect of D-penicillamine on the accumulation of exudate cells in the intraperitoneally implanted gelatin sponges

<table>
<thead>
<tr>
<th>Group no./treatment</th>
<th>Number of exudate cells</th>
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<tr>
<td></td>
<td>Time after implantation</td>
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<td>6 h</td>
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Sensitised animals

Ferritin sponge implants

1. Control no treated 80.1±8.6 154.1±7.4 229.5±11.4 162.3±9.8 143.2±8.7
2. D-penicillamine treated (orally) 108.0±6.7* 195.1±8.1*** 265.2±10.9* 189.3±7.6* 148.2±7.6
3. D-penicillamine treated (orally) 109.2±6.9* 196.4±12.6** 285.6±15.7* 211.2±13.9* 174.6±11.9*
4. D-penicillamine treated (orally) 113.6±9.2* 212.8±12.1** 274.8±14.9* 205.2±12.2* 162.4±9.9

PBS sponge implants

5. Control no treated 90.1±7.2 99.1±6.0 72.4±3.7
6. D-penicillamine treated (orally) 85.8±4.7 98.4±6.4 76.5±6.9

Nonsensitised animals

Ferritin sponge implants

7. Control no treated 33.8±3.8 75.7±5.8 90.0±6.9 94.6±6.4 85.7±4.5

Each group consisted of 4 guinea-pigs. *D-penicillamine administered for 14 days before sensitisation. †D-penicillamine administered for 14 days before challenge. ‡D-penicillamine administered for 30 days before challenge. §D-penicillamine administered for 14 days before gelatin sponge implantation. Results were expressed as mean counts per 0.25-mm² of 40 sections from 4 sponges ±SE. *p<0.05, **p<0.01 versus control group 1.

Table 2 Effect of D-penicillamine on the accumulation of polymorphonuclear and mononuclear cells in the intraperitoneally implanted gelatin sponges

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Exudate cell count analysis</th>
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<td></td>
<td>Time after implantation</td>
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</tbody>
</table>

Sensitised animals

1 Poly 70.5±7.6 124.8±6.0 167.5±8.6 66.5±4.8 45.8±4.2
Mono 9.6±1.4 29.3±2.0 62.0±3.8 95.8±5.4 97.4±5.9
2 Poly 100.0±6.7** 161.4±9.2** 199.5±12.2* 88.7±8.3* 53.3±4.0
Mono 7.5±0.8 33.7±2.8 65.7±4.7 101.1±3.6 95.0±5.7
3 Poly 101.2±6.6** 158.4±9.6** 186.4±12.7 79.4±7.6 46.0±3.6
Mono 8.0±1.2 38.0±4.3 99.2±4.8** 131.8±8.3** 128.6±8.8**
4 Poly 106.8±9.2** 173.6±12.2** 190.2±14.1 80.4±6.0 42.4±7.1
Mono 6.8±1.9 39.8±4.5 54.8±7.7* 124.8±10.4* 120.0±8.8*

Nonsensitised animals

7 Poly 29.1±3.2 57.5±4.4 61.9±3.4 40.7±2.8 34.3±2.8
Mono 4.7±0.5 18.2±4.4 28.1±2.4 53.9±4.3 51.4±2.4

See Table 1 for each group. Results were expressed as mean counts per 0.25-mm² of 40 sections from 4 sponges ±SE. *p<0.05, **p<0.01 versus control group 1.

The number of exudate cells in the ferritin sponges of ferritin-sensitised guinea-pigs, counted per 0.25-mm² of sections, reached its peak 24 hours after implantation of the ferritin sponges and decreased thereafter (Table 1). When D-penicillamine was administered before the sensitisation or challenge periods, the number of exudate cells in the ferritin sponges increased, whereas the number of cells in the PBS sponges did not increase. After the material was stained with haematoxylin and eosin, the morphology of the nuclei of the cells in the peritoneal exudate that had accumulated in the implanted gelatin sponges could be used to differentiate polymorphonuclear cells (PMN) from mononuclear cells (MN). The exudate cells that accumulated in the ferritin sponge (total numbers given in Table 1) were thus differentiated and counted separately (Table 2). When D-penicillamine was administered prior to the sensitisation or challenge periods, it resulted in an increase in PMN cells in the ferritin sponges in the ferritin-sensitised guinea-pigs within 6–12 hours of their implantation. However, only when the D-penicillamine was administered prior to challenge did it result in an increase in MN cells within 24 hours of implantation. Moreover, MN cells continued to increase at 48 and 72 hours following implantation, eventually exceeding the PMN cells and dominating the exudate cells in the ferritin sponges in the ferritin-sensitised guinea-pigs.
Discussion

It has been reported\(^2\)\(^3\) that administration of D-penicillamine results in an increased response to delayed hypersensitivity caused by Bordetella pertussis vaccine in rats. In the present experiment on guinea-pigs, when D-penicillamine was administered daily in a high dose\(^4\)\(^5\) of 200 mg/kg for 14 days before sensitisation or for 14 or 30 days before challenge, an enhancement of the delayed hypersensitivity reaction under the gelatin sponge implantation was observed. However, the results of examination of serial changes in the cell count based on an analysis of cells in the exudate indicated that MN accumulation markedly increased within 24 hours of implantation only when D-penicillamine was administered before challenge. D-penicillamine, when administered over a short duration,\(^2\)\(^6\) may be less effective in increasing the accumulation of MN. In either case the effect of D-penicillamine when administered before sensitisation differs from that when it is administered before challenge. This difference in the effects of D-penicillamine may be due to the fact that, while D-penicillamine does not act on T lymphocytes that have not recognised antigen, it does act on T lymphocytes that have recognised antigen after sensitisation, and as a result it enhances the chemotactic response of MN by reinforced lymphokines.

D-penicillamine administered before sensitisation or before challenge enhanced the chemotactic response on PMN in the early stages, at 6–12 hours, yet at 24–72 hours the percentage of PMN decreased as a result of the increase in MN. And the degree of the early (6–12 hours) PMN component bears no quantitative relationship to the magnitude of the subsequent (24–72 hours) MN accumulation. Though the delayed hypersensitivity reaction following gelatin sponge implantation has a prominent early (PMN) phase that recedes and is followed by a prominent delayed (MN) phase, it may be that the chemotaxis of PMN\(^7\) proceeds by a mechanism or through a pathway that is distinct from that of the chemotaxis of MN\(^8\) (counted as both macrophages and lymphocytes). Then as a result it is suggested that in the early stages D-penicillamine does act specifically on immunological agents or effector cells that mediate the chemotaxis of PMN.

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References