PREVENTIVE AND CURATIVE EFFECTS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON EXPERIMENTAL PERITONEAL INFLAMMATION IN MICE

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Abstract—Protein content of the peritoneal fluid of mice with induced inflammation was determined using a biuret reagent. The amount of protein in the peritoneal fluid was maximal at two hr after the i.p. injection of 1.2% acetic acid solution when the content of protein was tenfold that of normal mice. The increase in protein content in the peritoneal fluid corresponded well with the dye content leaked from the vascular bed. The anti-inflammatory drugs tested were curative as well as preventive on the increased vascular permeability.

Artificial peritoneal inflammation induced in mice or rats has been utilized to test the anti-inflammatory effect of drugs. As the vascular permeability increases early in the inflammatory process, the amount of dye-labelled plasma protein which exudes into the peritoneal fluid has been determined to estimate the intensity of inflammation (1-3).

The present study was performed to estimate the intensity of peritoneal inflammation by direct measurement of exuded plasma protein using a biuret reagent. The preventive and curative effects of anti-inflammatory drugs on the increased vascular permeability were also studied utilizing the present technique.

MATERIALS AND METHODS

Experimental animals

Five week-old male mice of ddY strain, weighing 25 to 30 g, were used in the present experiment. The mice were kept in metal cages in a constant temp. (24 ± 2°C) and humidity (50 to 70%). A cube diet (CLEA, Japan Inc., Tokyo) and water was provided ad libitum.

Irritation

To induce an inflammatory reaction each animal was administered i.p. 0.25 ml of 1.2% acetic acid, unless otherwise stated.

Determination of protein in the peritoneal fluid

Mice were sacrificed three hr after the injection of acetic acid. Abdominal walls were excised widely so as to expose the viscera. The exposed portions were then irrigated in a Petri dish with 5 ml of saline which contained 0.1 mM disodium ethylenediaminetetraacetate to inhibit coagulation of washings. The volume of each washing was made up

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to 6 ml with saline, then centrifuged at 1500 g for 10 min. The total amount of protein in the supernatant fluid was determined with the biuret reagent according to the method of Gornall et al. (4). Hemorrhagic exudate which was induced by the thrust of a needle through a blood vessel when acetic acid was injected i.p. was discarded.

Estimation of the anti-inflammatory drugs

To estimate the preventive effect of the test drugs on inflammation, mice were given drugs by oral administration 30 min before acetic acid injection. Curative effects of several drugs on inflammation were estimated by oral administration of drugs one hr after the irritant injection. Five mice were used for each dose. In this case, drugs which had been emulsified in 2% solution of gum arabic were administered. The vehicle only was given to control mice. Irritation of the animal and determination of the protein content in the peritoneal fluid followed the method mentioned above.

RESULTS

Protein exudation in the peritoneal fluid of inflamed mice

In order to determine the process of protein accumulation in the peritoneal fluid, mice were sacrificed at various times after the irritant injection.

As is seen in Fig. 1, the protein content in the peritoneal fluid gradually increased after the injection of 0.6% or 1.2% acetic acid solution, and reached a maximum approx. two hr after when the amount of protein was ten times that in normal mice. When animals were injected with 0.6% acetic acid, the content of exuded protein was half that of the mice which had been given 1.2% acetic acid injection. In this case, the peak of the protein content appeared earlier than in the case of those injected with the 1.2% acetic acid solution.

Fig. 1. Time course of protein exudation in the peritoneal fluid of mice which had been injected with 0.6 or 1.2% acetic acid. Each point represents the mean±S.E.

△ : 0.6% acetic acid, ● : 1.2% acetic acid
To determine the detailed relationship between the acetic acid concentration administered and the content of the protein exuded, mice were injected with acetic acid in concentrations ranging from 0.4 to 1.2% and sacrificed 3 hr after. As shown in Fig. 2, the protein content increased as the injected irritant became concentrated.

**Fig. 2.** Relationship between the concentration of acetic acid which had been injected into the peritoneal cavity of mice and the corresponding protein exudation. Protein was determined 3 hr after the acetic acid injection.

**Fig. 3.** Correlation between exudation protein content and exuded dye content in the peritoneal fluid of mice which had been injected i.p. with graded concentrations of acetic acid.

*Correlation between the exuded protein content and the leaked dye content in the peritoneal fluid*

In order to determine the correlation between the increase in protein content in the peritoneal fluid and the change in vascular permeability, mice were injected i.v. with 0.1 ml of 1% Evan’s blue just prior to the injection of graded concentrations of acetic acid. Mice were sacrificed three hr after the irritant injection and the contents of protein and dye in the peritoneal fluid were measured. One half of the supernatant fluid of the washings was used for the determination of protein and the other half for dye content. The total amount of dye leaked in the supernatant fluid of the washings was determined by colorimetry. The result, as is shown in Fig. 3, indicates a linear correlation between the contents of protein exuded and the dye leaked. This apparently indicates that the increased protein in the peritoneal fluid derived from the plasma protein.

*Preventive effect of anti-inflammatory drugs on peritoneal inflammation*

Time course of protein content in the peritoneal fluid of mice orally administered 300 mg/kg of aspirin and that of control mice is shown in Fig. 4. Aspirin showed an inhibitory effect on the increase in protein content in the peritoneal fluid. The inhibitory effect of aspirin was recognized at an earlier stage (at 30 min and 1.5 hr after the irritant injection) as well as at later stage of the inflammation (at 2.5 hr and 3.5 hr after the
irritant injection). Preventive effects of anti-inflammatory drugs are summarized in Table 1. The increase in protein content was prevented by all the drugs tested herein.

**TABLE 1. Preventive effect of anti-inflammatory drugs on protein exudation in peritoneal fluid of mice.**

| Drug            | Dose (mg/kg p.o.) | Protein content (mg) | Inhibitory ratio (%) |
|-----------------|-------------------|----------------------|----------------------|
| Control         |                   | 22±1.6               | 40.0                 |
| Aspirin         | 100               | 13±1.8**             | 63.6                 |
|                 | 300               | 8±0.8**              |                      |
| Phenylbutazone  | 100               | 16±1.2*              | 27.3                 |
|                 | 300               | 13±0.4**             | 40.9                 |
| Control         |                   | 30±1.8               |                      |
| Mefenamic acid  | 100               | 16±1.6**             | 46.7                 |
|                 | 300               | 12±1.2**             | 60.0                 |
| Control         |                   | 26±1.7               |                      |
| Indomethacin    | 1                 | 17±1.3**             | 34.6                 |
|                 | 5                 | 11±1.4**             | 57.7                 |
| Control         |                   | 30±1.4               | 23.3                 |
| Ibuprofen       | 300               | 23±1.4**             |                      |

Drugs were orally administered 30 min prior to i.p. injection of 0.25 ml/body of 1.2% acetic acid. Protein was determined 3 hr after the acetic acid injection.

Results are shown as means±S.E.

* statistically significant at P<0.05

** statistically significant at P<0.01

Fig. 4. Preventive effect of aspirin on protein exudation in the peritoneal fluid of mice which had been injected with 1.2% acetic acid. Aspirin was administered per os 30 min before the acetic acid injection. Each point represents the mean±S.E.

○ control, ● aspirin.
Curative effect of anti-inflammatory drugs on peritoneal inflammation

Mice were orally administered 300 mg/kg of aspirin one hr after the irritant injection. As is shown in Fig. 5, aspirin reduced the protein content which had increased, to about half that of the control mice two hr after the drug administration, but the effect of aspirin was not recognized 30 min after the drug administration.

Curative effects of other drugs are summarized in Table 2. All the drugs tested showed a significant curative effect.

![Fig. 5. Curative effect of aspirin on protein exudation in peritoneal fluid of mice which had been injected with 1.2% acetic acid. Aspirin was orally administered 1 hr after the acetic acid injection.](image)

**Table 2. Curative effect of anti-inflammatory drugs on protein exudation in peritoneal fluid of mice.**

| Drug         | Dose (mg/kg p.o.) | Protein content (mg) | Inhibitory ratio (%) |
|--------------|-------------------|----------------------|----------------------|
| Control      |                   | 22 ± 2.1             |                      |
| Aspirin      | 300               | 10 ± 0.6*            | 54.5                 |
| Control      |                   | 27 ± 1.9             |                      |
| Phenylbutazone | 100             | 16 ± 1.8*            | 40.9                 |
| Mefanamic acid | 100              | 17 ± 2.3*            | 37.1                 |
| Control      |                   | 26 ± 1.7             |                      |
| Indomethacin | 5                 | 8 ± 0.7*             | 69.1                 |
| Control      |                   | 22 ± 2.01            |                      |
| Ibufenac     | 300               | 2 ± 1.4*             | 45.5                 |

Drugs were administered per os 1 hr after an i.p. injection of 0.25 ml/body of 1.2% acetic acid. Protein was determined 2 hr after drug administration.

Results are shown as means ± S.E.

* statistically significant at P < 0.01
DISCUSSION

Using mice, the protein content in the inflamed peritoneal fluid produced by irritation was measured with a biuret reagent. The present technique employed is considered to be advantageous for screening anti-inflammatory activity of compounds, since the intravenous injection of dye can be preserved and the result of the experiment is highly reproducible.

The increased protein in the peritoneal fluid after irritation appears to be derived from the plasma, as the amount of protein increase corresponded well with that of the dye leaked from the vascular bed. This was to be expected, as it has been reported, that the injected dye easily combines with the circulating plasma protein, particularly with albumins (5).

It was also ascertained in the present study that most of the anti-inflammatory drugs were not only preventive but also curative on the increased vascular permeability.

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