ANTIULCEROGENIC PROPERTY OF SODIUM POLYACRYLATE ON EXPERIMENTAL ULCERATIONS IN RATS

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Abstract The antiulcerogenic properties of sodium polyacrylate (PAS) against experimental ulcerations in rats were studied and compared with amylopectin sulfate (APS). Intragastric administration of PAS or APS to the pylorus ligated rat reduced ulceration. The protective effects of both drugs were dose dependant and PAS was less effective than APS. The gastric lesion induced by reserpine was inhibited by the oral ingestion of PAS mixed with sugar, while APS had no protective effect. More ingesta were found in the stomachs of the PAS group than the APS or control groups. PAS was confirmed to be an inhibitor of pepsin in vitro, but less effective than APS. Although PAS had little effect on the secretory volume of the gastric juice of the 6 hr pylorus ligated rat, it did cause a reduction of free acid in the juice. PAS did not possess anticoagulant activity.

It has been observed that sodium polyacrylate (PAS) possesses protective effects on the gastroesophageal ulcers in swine (unpublished). In this report we describe the antiulcerogenic activity of PAS on experimental ulcers in the rat.

As PAS is a high molecular and polyanionic substance, experiments were carried out to compare this drug with amylopectin sulfate (APS), a known antiulcerogenic, which also has high molecular polyanion (1). As APS has a strong anticoagulant activity, the effect of the drug on coagulation was also studied.

MATERIALS AND METHODS

Antiulcerogenic Activities

Pylorus ligated rat: The inhibitory activities of PAS and APS against the ulcers induced in rats with the pylorus ligations described by Shay et al (2) were studied. Groups of 4 or 5 male Wistar rats, weighing 250-300 g, were utilized following a fast of 48 hr. The surgery was carried out under ether anesthesia. A small incision was made just below the gastroesophageal sphincter. A capsule containing 25-100 mg of the test substance was inserted into the stomach through the incision. Rats in the control group were given an empty capsule. After 19 hr, the rat was sacrificed and the stomach removed. The gastric mucosa was examined under a ten times magnification. The degree of gastric ulceration was estimated by the method of Radwan and West (3). Ulceration of a stomach was given a score from 0 (normal) to 4 according to its severity. When the animal died from forestomach perforation within 19 hr after the ligation, one was added to the score. The maximum score for each animal was therefore 5. The
mean ulcer score for each group of rats was calculated and then multiplied by the percentage of ulcerated animals in the groups, to give the ulcer index.

**Reserpine ulcer:** The lesions were also induced in rats by the method of Adami et al (4). Groups of 4 or 5 male Fischer rats, weighing 150-200 g, were used. After a fast of 24 hr, rats were allowed to eat granulated sugar alone or sugar plus the test substances for 8 hr. The rats were administered 5 mg/kg of reserpine i.p. thereafter. Either 8 hr or 20 hr after the injection, the rats were sacrificed and the stomachs removed with ingesta and weighed. The gastric mucosa was examined under a 10 times magnification. Lesions were found in the glandular portion of the stomach and were expressed as ulcer index which was estimated by the method of Kelly and Robert (5). The percentage of animals with lesions was determined, severity of the lesion was graded according to a scale from 0 (normal) to 4 and the number of ulcers per rat was recorded. An ulcer index was calculated by adding the incidence of ulceration (divided by 10), average severity of the ulcers and the average number of the ulcers per rat.

**Stress ulcer:** The stress ulcer was produced by the Takagi and Okabe's method (6). Groups of 3 or 4 male Wistar rats, weighing 220-260 g, were used. Rats fasted for 24 hr were given the test substances mixed with granulated sugar for 8 hr and then restrained in a cage. The cage was immersed vertically into a water bath up to the height of the xiphoid process. The bath was kept at 23°C for 20 hr. The degree of gastric ulceration was determined using the same system as in the reserpine ulcer group described above and the stomach weight was measured.

**Gastric Secretion**

The pylorus ligated rats were prepared by the same method described above. Six hr after ligation, the volume of gastric juice was measured in a cylinder and pH was determined by a pH-meter. The titrations were made with 0.1 N NaOH using an automatic titrator (TOA Electronics LTD, HS-1B). Free acid was defined as the acid titratable to pH 2.9 and total acid was the acid titratable to pH 8.3. Pepsin activity in 0.05 ml of gastric juice was measured using casein as the substrate. The end product was measured at 280 m\(\mu\), and the result was expressed as optical density reading.

**Antipeptic Activity in Vitro**

The in vitro antipeptic properties were measured by a modification of the casein digestive method of Kunitz (7). Bovine pepsin was incubated at 37°C for 30 min at pH 2 with casein alone or casein plus PAS or APS. The concentration of pepsin used was such that an optical density of about 0.58 at 280 m\(\mu\) was obtained when incubated with 1% casein substrate. Equal amounts of PAS or APS were added to the control tubes after incubation. The reaction was stopped by 10% trichloroacetic acid. The samples were then centrifuged and the optical density of the supernatant was measured spectrophotometrically at 280 m\(\mu\). The effects of PAS and APS were determined for several concentrations varying from 0.1 to 1.0 mg/ml.

**Anticoagulant Activity**

Anticoagulant activity of PAS was determined in vitro. Coagulation time, measured
by the method of Lee and White (8) during incubation at 37°C, was determined. Concentration of PAS, APS and heparin varying from 0.02 to 0.1 mg/ml of blood was tested.

**Drugs**

Drugs used in this study were: sodium polyacrylate (PAS) (NIPPON KAYAKU CO., LTD), amylopectin sulfate (APS) (NIPPON KAYAKU CO., LTD), crystalline pepsin (TOKYO KASEI CO., LTD) and heparin (NOVO INDUSTRI, A/S).

**RESULTS**

**Antiulcer Studies**

*Pylorus ligated rats:* PAS and APS were tested at intragastric doses ranging from 25 to 100 mg per rat. Sixteen out of 19 animals in the control group developed forestomach ulcers and four rats among them died within 19 hr, showing signs of severe peritonitis. No animal in the test groups (60 animals), on the other hand, died during the test. As shown in Table 1, both substances produced the positive reductions in gastric ulceration. The ulcer index in PAS 100 mg group was the same as that in APS 50 mg group.

| TABLE 1. Inhibitory effects of intragastric PAS and APS on gastric ulcer in 19 hr pylorus ligated rats. |
|-------------------------------------------------|-------------------------------------------------|---------------------------------|-----------------|------------------|
| Treatment | No. of expts. | Rats with ulcers (%) | Ulcer scores | Ulcer index |
|-----------|----------------|-----------------------|--------------|-------------|
| Control   | 19             | 84                    | 57/19=3.0    | 251         |
| PAS       | 25 mg          | 10                    | 80           | 19.10-1.9  |
|           | 50 mg          | 10                    | 40           | 9.10-0.9   |
|           | 100 mg         | 10                    | 30           | 5.10-0.5   |
| APS       | 25 mg          | 10                    | 50           | 13.10-1.3  |
|           | 50 mg          | 10                    | 30           | 6.10-0.6   |
|           | 100 mg         | 10                    | 10           | 1.10-0.1   |

| TABLE 2. Effects of ingested PAS and APS on reserpine induced gastric lesion in rats. |
|-------------------------------------------------|-------------------------------------------------|---------------------------------|-----------------|------------------|
| Treatment (drug intake)* | Hr after reserpine | No. of rats | Weight of stomach (g) | Rats with lesion (%) | Severity per rat | Lesion per rat | Ulcer index |
|--------------------------|-------------------|-------------|----------------------|---------------------|-----------------|---------------|-------------|
| Control                  | 8                 | 15          | 1.0                  | 80                  | 2.3             | 3.0           | 13.3        |
| PAS (660 mg)             | 8                 | 15          | 3.2                  | 20                  | 0.5             | 0.5           | 3.0         |
| APS (580 mg)             | 8                 | 15          | 1.2                  | 73                  | 2.0             | 2.6           | 11.9        |
| Control                  | 20                | 15          | 1.3                  | 70                  | 1.9             | 1.8           | 10.7        |
| PAS (620 mg)             | 20                | 15          | 1.7                  | 60                  | 1.1             | 0.9           | 8.4         |

* Drug intake was calculated from the amount of the ingested granulated sugar in which the drugs had been mixed.

**Reserpine ulcer:** Results of a comparative study of the antiulcer activities of PAS and APS on the glandular lesions induced by reserpine are shown in Table 2. In the 8
hr test, APS was ineffective in preventing the lesion at an oral dose of 580 mg per rat, while a dose of 660 mg PAS produced significant inhibition. In the 20 hr test, PAS (620 mg) was ineffective in preventing lesions. A considerable amount of residue of PAS ingested was detected in the stomach 8 hr after reserpine administration.

Stress ulcer: After the restrained rats had been immersed for 20 hr, evident glandular lesion was present in most animals of the three groups (Table 3). Any protective effects of PAS or APS were absent. The ingested granulated sugar mixed with PAS or APS was not found in the stomach after 20 hr of restraint.

| Treatment (drug intake) | No. of rats | Weight of stomach (g) with contents per rat | Rats with lesion (%) | Severity per rat | Lesion per rat | Ulcer index |
|------------------------|-------------|--------------------------------------------|---------------------|-----------------|---------------|-------------|
| Control                | 9           | 1.0                                        | 100                 | 2.8             | 4.1           | 16.9        |
| PAS (550 mg)           | 10          | 1.3                                        | 90                  | 3.0             | 4.0           | 16.0        |
| APS (600 mg)           | 9           | 1.4                                        | 90                  | 2.7             | 4.9           | 16.6        |

Table 4. Effects of PAS and APS on the gastric secretion in the 6 hr pylorus ligated rats.

| No. of rats | Volume ml | pH | Free acid (0.1 N NaOH) | Total acidity | Antipeptic activity 280 ml |
|-------------|-----------|----|------------------------|---------------|---------------------------|
| Control     | 10        | 8.0±1.9 | 1.2±0.2 | 5.2±1.6 | 7.6±2.0 | 0.90±0.24 |
| PAS 25 mg   | 10        | 8.6±3.1 | 1.5±0.9 | 4.9±3.5 | 7.8±3.9 | 0.72±0.32 |
| 100 mg      | 10        | 9.1±2.2 | 1.8±0.6* | 1.9±1.8** | 4.6±2.2* | 0.50±0.24** |
| APS 25 mg   | 10        | 8.5±4.3 | 1.0±0.4 | 5.4±4.7 | 7.5±5.5 | 0.34±0.17** |
| 100 mg      | 10        | 9.2±4.8 | 1.3±0.2 | 3.8±4.7 | 6.1±2.2 | 0.35±0.06** |

mean±SD, *P<0.05, **P<0.01

Gastric Secretion

The effects of PAS and APS on the gastric secretions of 6 hr pylorus ligated rats are shown in Table 4. The mean volumes of gastric juice did not differ among the three groups, whereas the free (P<0.01) and total acidities (P<0.05) were significantly reduced in the PAS 100 mg group. Free acidity was also significantly (P<0.05) reduced in the APS 100 mg group. Antipeptic activities were present to a varying extent in the PAS 100 mg, and APS 25 mg and 100 mg groups.

In Vitro Antipeptic Activity

The comparative study of antipeptic activity of PAS and APS is summarized in Table 5. APS displayed a higher activity than PAS.

Anticoagulant Test

As shown in Table 6, APS possessed a heparin-like quality in prolonging the clotting time of whole blood in vitro while PAS did not.
**DISCUSSION**

Since an antipeptic activity of chondroitin sulfate in dogs was reported by Babkin and Komarov (9), many sulfated polysaccharides have been observed to have antipeptic activity (10, 11). Subsequently, degraded carrageenan (12), amylopectin sulfate (1), and other sulfated polysaccharides (13, 14) display antiulcerogenic activities against the experimentally induced ulcers.

In the experiments herein, it was demonstrated that PAS a) prevents forestomach ulceration, b) may prevent glandular ulceration, c) inhibits the proteolytic action of pepsin, d) decreases the free acid in the stomach, e) does not inhibit the volume of gastric secretion and f) does not prevent blood coagulation. The first three effects are also characteristic of sulfated polysaccharides. PAS is a high molecular polyanionic substance (MW>2,000,000), and when dissolved in water, produces a solution of high viscosity. Sulfated polysaccharides are also high molecular and polyanionic substances, the water solutions of which are also highly viscous.

The mechanisms of the antiulcerogenic activities of the sulfated polysaccharides have been discussed by many authors. Bianchi and Cook (1) concluded that the preventive effect of APS against the forestomach ulcer in the rat was mainly due to the inhibition of pepsin, either as the result of the combination between the enzyme and the polysaccharide resulting in a complex with less activity or to a reduction in the amount of free pepsin available at any one time. Sun (15) postulated that APS might form a new complex with gastric mucous, increasing its viscosity and bestowing antipeptic property upon it, and this reinforced mucous barrier would enhance a protective effect. Anderson and
Watt (12) suggested two main actions of degraded carrageenin on the histamine ulcer in guinea pig: it reacted possibly with mucoprotein to form a complex, thus it served to enhance the physical protection afforded by mucin and the polysaccharide conferred antipeptic effects on this complex.

The antipeptic activity of PAS may result from the coagulations of the substrate and the enzyme since the polyanionic substance generally make a complex with a protein by means of ionic or hydrogen bond. The depression of the free acid of the gastric juice may not due to the antisecretory action of PAS because it failed to decrease the volume output. HCl in the gastric juice may be neutralized by PAS.

Bianchi et al (1) reported the protective effect of APS on the glandular lesion induced by cortisone in the rat. They administered the drug three times a day or put in into the drinking water given the fasted rat. On the other hand, we administered the drugs by means of feeding the rat granulated sugar mixed with the drugs in order to produce the ulcer in non-fasted animals. In the 8 hr reserpinized rats, evident lesions in the glandular portions were produced in the APS or control groups, while in the PAS group, lesions were relatively rare. Furthermore, in the PAS group the gastric content was greater than in the other groups (Table 2). The gastric remains of 20 hr reserpinized or stressed (20 hr water immersion) rats were rarely found and the antiulcerogenic property of PAS was seldom observed (Tables 2, 3). These findings indicate that the presence of the drug in the stomach may be needed for its antiulcerogenic activity.

Consequently, it is considered that PAS reduces the digestive activity of gastric juice by the inhibition of pepsin, reduction of free acid and prolongation of the gastric emptying.

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