UDC 615.7[612.115.35]-092

29. Masaya Namekata : Studies on Oxidized Starch Sulfates for Medical Purposes. IV.[1] Protective Effect of Sulfates of Oxidized Starch and its Reduced Products on Experimental Peptic Ulceration.

(Research Laboratory, Chugai Pharmaceutical Co., Ltd.[*1])

In the preceding report,[1] the inhibitory action of oxidized starch sulfate and sulfate of reduced product of oxidized starch on pepsin proteolytic action was reported. In the present work, protective effect of oxidized starch sulfate or reduced product of oxidized starch on experimental peptic ulcer was examined to investigate their prophylactic or medical use. The samples of various sulfur content and intrinsic viscosity were used to investigate their protective effect on the pylorus-ligated rat.

Materials and Methods

Oxidized starch sulfate and sulfate of reduced product of oxidized starch were the same as those used in the previous work.[1] Dextran sulfate, heparin, chondroitinsulfuric acid, glycyrrhiza extract, aluminium hydroxide, L-methionine methylsulfonium chloride (Vitamin U) were subjected to this experiment. Healthy male Donryu strain rats (150~270 g.), kept in an air-conditioned room at 23~25° for more than 3 days, fed on compressed food, were used.

Experimental Ulceration by the Ligation of Pylorus[2]——Each animal was housed in a separate cage and starved for 47 hr. before the operation. Under Et_2O anaesthesia, an abdominal midline incision was made, extending from the xiphoid in a distance of about 1 cm. The pylorus was then ligated with a silk thread, taking care not to damage other internal organs, especially the duodenum. Immediately after the operation, the sample solution or water was administered through the esophagus by a stomach tube and animals were deprived of food and water for 18 hr. The operated rats were designated as the "Shay rat." Under $CHCl_3$ anaesthesia, the abdomen was opened, the stomachs ligated at the cardiac orifice, was removed, and gastric juice was transferred into a graduated centrifuge tube. The stomach was opened along the greater curvature and the degree of ulceration was determined with naked eye, by examining the number and size of ulcer on the mucous membrane.

Analysis of Gastric Content——Gastric content was centrifuged at 2000 r.p.m. for 10 min. and the volume of supernatant and of solid was recorded. To 2 cc. of the gastric juice, 0.5% EtOH solution of dimethylaminoazobenzene and 1% phenolphthalein were added as the indicator, and titrated with 0.02*N* NaOH. The acidity was expressed in cc. of titer. The samples were prepared by diluting 2 cc. of gastric juice to 10 or 20 volumes with distilled water and adjusting to pH 1.6 with HCl. The peptic activity was determined in the same way as in the previous paper,[1] according to the method of Bonfil, *et al.*,[3] and expressed as the mg. of tyrosine liberated per 1 cc. of gastric juice. pH of gastric juice was determined with a pH meter.

Experimental Results

I. Reference Experiment——Wistar strain rats were used for these experiments by Shay and other research workers.[2,4~6] In the present experiment, Donryu strain rats were used and it became necessary to find the percentage of gastric ulcer occurrence in this strain. The ulceration on Donryu strain (150~270 g.), after 18 hr. of pylorus ligation, was distinctly observed on 24 out of 25 animals or in 96%, but the ulcers produced were various in number and size in each animal. For example, 43 ulcers were produced in the most affected one, and 7 in the least cases, and the average was 16.4.

*1 Takadaminami-cho, Toshima-ku, Tokyo (行方正也).

1) Part III : This Bulletin, **10**, 171(1962).
2) H. Shay : Gastroenterology, **5**, 43 (1945).
3) S. Bonfils, M. Dubrasquet, A. Lambling : Rev. franc. etudes clin. et biol., **5**, 71 (1960).
4) H. Arae : Nihon Shôkaki-Byô Gakkai Zasshi, **54**, 731 (1957).
5) H. Nomura : Fukuoka Igaku Zasshi, **50**, 354 (1959).
6) S. Levey, S. Sheinfeld : Gastroenterology, **27**, 625 (1954).

The largest ulcer was 5 cm. in diameter, smallest one being 1 mm., and holes were observed on gastric mucous membrane in four of the animals. The volume of supernatant of gastric juice was from 7 to 15 cc. and the solids volume was 0.6~0.1 cc. The volume of gastric content was different in each case, being almost proportional to the weight of rats, while the percentage of ulcer induction was independent of the weight of rats. From these facts, it was confirmed that the ulcer was produced in Donryu strain rats in as high a percentage as in the Wistar strain.

II. Protective Effect of Oxidized Starch Sulfate and Sulfate of Reduced Product of Oxidized Starch on Peptic Ulceration——Protective effect of the sulfate (Compound No. ROS26-S. 4) of a reduced product of oxidized starch, having a strong inhibitory activity on pepsin *in vitro*, was examined with six groups of Shay rats (170~190 g. in body weight). The animals were examined in the following five groups and the sample solution was administered soon after the pylorus ligation in a dose of 5, 10, 20, 30, 40 mg. of the sample per animal. The control group was given 0.4 cc. of water. The stomach was removed 18 hr. after administration of the test preparation, the inner surface was examined, and the gastric juice was analyzed. The results are shown in Table I. It was found that the ulceration was 100% in the control group of the five of the operated rats which received

Table I. The Effect of Oxidized Starch Sulfate (ROS26-S. 4) on Shay Rats Ulcers and on their Gastric Juice Pepsin Value

No. of rats	Doses (mg.)	Ulcer		Gastric juice			
		Ave. No./rat	No. of animals/group	Vol.	pH	Total acid	Pepsin (as mg. tyrosine)
5	0	31	5	11.7	1.4	72.4	24.2
5	5.0	8	4	10.8	1.1	81.3	22.1
5	10.0	2	2	11.8	2.1	86.5	16.9
5	20.0	1	1	9.1	1.2	71.6	7.1
5	30.0	0	0	12.2	1.4	60.0	5.6
5	40.0	0	0	12.0	1.6	67.3	4.6

water alone. A considerable protection from ulceration was observed in the groups treated with ROS26-S. 4 and the percentage of ulcer production in the groups treated with 5, 10 and 20 mg. of it was 80%, 40% and 20%, respectively. The number of the ulcers was also decreased with increasing dosage and no ulceration was observed in the group treated with 30 and 40 mg. of the preparation. There was no difference in gastric juice among these groups, such as the volume of supernatant and of solid, pH, and total acid, but the loss of pepsin activity was proportional to the increase of dosage, and it was marked especially in the groups treated with more than 10 mg. of the preparation. As shown in Fig. 1, a correlation was found between the loss of pepsin activity in gastric juice and protective effect of the sulfates on ulceration in Shay rats.

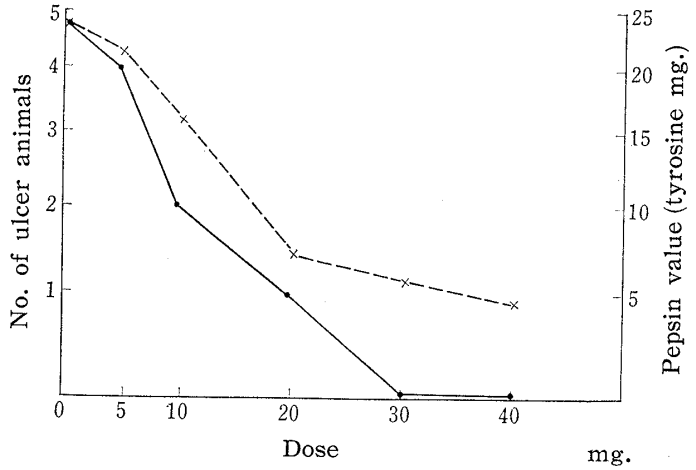

Fig. 1.

×------× Pepsin value
•——• No. of ulcer animals

In the previous paper,[1] it was shown that the inhibitory action of OS-S and ROS-S on crystalline pepsin *in vitro* depended mainly on the sulfur content. Therefore oxidized starch sulfate and sulfate of reduced product of oxidized starch with various sulfur contents and viscosity, heparin, chondroitinsulfuric acid and dextran sulfate were administered 30 mg. per animal to each of rats in order to examine the correlation among the protective effect on ulceration, loss of pepsin activity

Table II. Effect of Oxidized Starch sulfates, Sulfates of Reduced Product of Oxidized Starch, Heparin, Chondroitinsulfuric Acid, and Dextran Sulfate on Ulcer Formation in Shay Rats

Compound No. *	S (mole)	[η]	Anticoagulant activity U/mg.	No. of rats	Ulcer		Gastric juice				
					Ave. No./rat	No. of animals /group	Vol.	pH	Free HCl	Total acid	Pepsin (as mg. tyrosine)
OS26-S. 7	0.94	0.007	0.6	5	11	5	12.5	1.5	39.0	73.0	18.2
OS27-S. 5	1.21	0.008	1.6	5	9.0	5	12.0	1.6	35.0	67.4	16.3
Distilled water				5	38.3	5	9.8	2.1	27.2	60.9	22.9
OS26-S. 1	0.55	0.007	0.3	5	6.0	5	10.5	1.2	64.7	102.5	17.8
OS26-S. 2	0.80	0.007	0.4	5	9.7	5	10.0	1.3	52.4	87.2	17.2
OS26-S. 3	0.94	0.007	0.5	5	4.7	4	13.0	1.3	53.9	84.6	16.2
OS26-S. 4	0.97	0.007	0.6	5	4.2	4	12.0	1.8	52.4	88.9	16.6
Distilled water				5	12.4	5	11.5	1.4	51.9	96.9	21.1
ROS26-S. F1	1.54	0.009	8.6	5	0.2	1	12.0	1.4	40.5	72.0	5.3
ROS26-S. F2	1.65	0.009	5.5	5	0	0	13.5	1.4	58.5	76.0	5.4
ROS26-S. F3	1.53	0.008	4.2	5	2	2	12.5	1.5	47.0	74.2	8.3
ROS26-S. F4	1.84	0.007	2.2	5	4	4	14.0	1.3	45.0	90.3	21.6
Distilled water				5	5	5	15.0	1.1	71.4	117	24.7
ROS26-S. 1	0.25	0.015	0.27	5	4.0	5	12.2	1.8	62.2	101.2	18.1
ROS26-S. 2	0.99	0.015	7.17	5	0.8	3	9.4	2.1	55.6	74.2	6.13
ROS26-S. 3	1.18	0.015	9.68	5	0	0	10.6	2.3	41.6	56.4	3.87
ROS26-S. 4	1.59	0.015	11.25	5	0	0	11.2	1.9	43.2	52.4	3.04
Distilled water				5	7.5	5	9.0	1.9	59.1	89.4	20.3
OS12-S. 3	1.68	0.010	6.2	5	8.7	4	13.0	2.4	49.5	83.7	17.6
OS17-S. 1	1.54	0.010	5.5	5	6.2	2	13.5	2.5	45.7	77.0	10.8
OS.Y-S. 1	1.04	0.012	16.7	5	7.1	3	13.5	2.0	62.4	93.6	18.2
ROS.Y-S. 1	1.89	0.034	42.4	5	1.4	1	14.0	2.3	60.9	88.5	9.1
ROS18-S. 1	1.75	0.039	27.7	5	0.8	0	12.1	2.1	66.0	93.6	4.7
Distilled water				5	12.6	5	13.5	2.2	41.5	45.4	23.7
Heparin	(9.41%)		126	5	2.3	3	14.0	1.4	50.1	92.4	16.05
Chondroitin-sulfuric acid	(6.6%)		0.3	5	10.3	5	13.5	1.1	73.1	109.0	25.8
Dextran sulfate	(17.8%)		29.5	5	0.3	1	14.2	1.3	42.9	72.2	6.0
ROS24-S. 4			33.2	5	0.2	1	11.0	1.5	35.0	67.8	9.6
Distilled water				5	26.3	5	13.0	1.1	71.8	108.4	24.4

* OS-S : Oxidized starch sulfate.
ROS-S : Sulfate of reduced product of oxidized starch.

in gastric juice, and the inhibitory action. These results are shown in Table II. It was found that the ulcer formation was 100% (number of ulcers per rat was more than 12) in the control group, but percentage of ulceration was 60~0% (number of ulcers per rat : 7.1~0) in the groups treated with the sample with more than 1.5 mole/glucose unit sulfur content and viscosity above 0.015 [η], and in the groups treated with the sulfates of less than 1 mole/glucose unit sulfur content and viscosity below 0.01 [η], incidence of ulceration was 100~80% (number of ulcers per animal being 9.7~4.2). There was an apparent difference in the occurrence of ulcers between control groups and sulfate-administered groups.

According to the gastric juice analysis, pepsin activity in the control group was 20.3~25.5 mg. as the liberated tyrosine, and 17.0~3.0 mg. of tyrosine in the treated group, and the loss of pepsin activity was marked in the groups treated with the sulfates of high sulfur content. It was also found that the protective effect on ulceration was proportional to the loss of pepsin activity in gastric juice, and the inhibitory action of the sulfates on pepsin in gastric juice agreed with that on crystalline pepsin *in vitro*.

There was almost no difference in the volume and pH of gastric content between the control and treated groups, but the total acidity of the latter was a little lower than that of the former. It was demonstrated that the sulfate of reduced product of oxidized starch of more than 1.5 mole/glucose unit sulfur content and viscosity [η] of 0.010~0.015, such as compound No. ROS26-S. 4 and ROS26-SFI, were the most effective for the protective effect on ulceration.

III. Comparison of Sulfate of Reduced Product of Oxidized Starch with Other Compounds——Protective effects of glycyrrhiza extract, aluminium hydroxide, and L-methionine methylsulfonium chloride (vitamin U) dissolved in water in a concentration of 100 mg./cc., in a dose of 30 mg. per animal was examined according to the procedure described above and compared with that of sulfate of reduced product of oxidized starch. The results are shown in Table III.

Table III. Comparative Effect of Sulfate of Reduced Product of Oxidized Starch and Other Substances on Ulcer Formation

Substances	Ulcer			Gastric juice				
	No. of rats	No./rat	No. of animals	Vol.	pH	Free HCl	Total acid	Pepsin (as mg. tyrosine)
L-Methionine methylsulfonium chloride	5	11.0	5	10.5	1.3	50.9	90.4	21.8
Aluminium hydroxide	5	18.8	5	7.2	3.2	7.0	55.0	24.0
Glycyrrhiza extract	5	38.6	5	9.6	2.9	9.0	60	20.3
ROS26-S. 4	5	0	0	11.0	1.9	43.2	41.2	7.4
Distilled water	5	30.8	5	9.0	3.6	59.1	62	25.5

It was noticed that the ulceration was 100% (number of ulcer per animal were 11.0～30.8) and pepsin activity in gastric juice was 21.8～24.0 mg. of tyrosine in the groups treated with these preparations. In the control group, ulceration rate was 100% (number of ulcers per rat being 16.5～30.8) and pepsin activity in gastric juice was 20.8～25.5 mg. as tyrosine. They had no protective effect on this experimental ulceration and no inhibitory activity on pepsin. In the group treated with sulfate of reduced product of oxidized starch, the ulceration rate was 0% (number of ulcers per animal was 0) and pepsin activity in gastric juice was 7.4 mg. as tyrosine. The anti-acidic effect of aluminium hydroxide and sulfate of reduced product of oxidized starch on HCl was examined, dissolving 30 mg. of sample into 10 cc. of HCl (pH 1.6). The pH of HCl changed to 2.1～2.6 by aluminium hydroxide, but there was no pH change by sulfate of reduced product of oxidized starch, showing no anti-acidic effect.

IV. Subcutaneous Administration of Sulfate of Reduced Product of Oxidized Starch——Ten mg. of a sulfate (compound No. ROS26-S. 4) of reduced product of oxidized starch, dissolved in 0.9% NaCl solution in a concentration of 100 mg./cc., was subcutaneously administered at 2 hr. intervals from 6 hr. before, immediately after, and 2 hr. after the ligation. The results, obtained according to the procedure described above, are given in Table IV. No difference was found in ulceration rate and pepsin activity in gastric juice between the control and the administered groups.

Table IV. Effect of Sulfate of Reduced Product of Oxidized Starch (administered subcutaneously) on Ulcer Formation in the Shay Rat

	Ulcer			Gastric juice			
	No. of rats	No./rat	No. of animals	Vol.	pH	Total acid	Pepsin (as mg. tyrosine)
ROS26-S. 4	5	11.7	5	11.5	1.2	117.5	24.0
Distilled water	5	9.3	5	9.0	1.3	87.5	26.2

V. Effect on Ulceration caused by Experimental Stress——Takagi, *et al.*[7] reported on experimental ulceration by stress on rats, that ulceration was produced by soaking the fixed rats in water at about 25°.

The effect of sulfate of reduced product of oxidized starch on stress ulceration was examined. First, the animals in groups of seven rats, were soaked into water in a fixed state, and compound No. ROS26-S. 4 was administered intra-esophageally in 30 mg./rat dose (concn., 100 mg./cc.). After 15 hr., the stomach was examined. There was no difference in the state of ulceration between the control and administered groups. Then the animals in groups of 15 rats received 20 mg. doses of compound No. ROS26-S. 4 (100 mg./cc.) intra-esophageally, six times at 2 hr. intervals. The control group was given 0.2 cc. of water at the same intervals. After 15 hr., the stomach was removed and examined. It was noticed that the ulceration appeared on all stomachs along the ridges of the mucosal folds and there was again no difference between the two groups. It seems that there is no protective effect of sulfate of reduced product of oxidized starch on ulceration of stress rats.

7) K. Takagi, Y. Kasuya, K. Watanabe: Paper presented at the 4th Kanto District Meeting of the Pharmaceutical Society of Japan, October, 1960.

Conclusion

The result of the present series of experiments shows that there is correlation between the ulceration rate and decrease in pepsin activity of gastric juice when treated with oxidized starch sulfate and sulfate of reduced product of oxidized starch.

Glycyrrhiza extract, aluminium hydroxide, L-methionine methylsulfonium chloride showed no effect on decreasing the pepsin activity, and have no protective effect on this experimental ulceration. No protective effect of sulfate of reduced product of oxidized starch was observed either on Shay rat when administered subcutaneously or on stress ulceration when subcutaneously and intra-esophageally administered, the latter of which is regarded as endocrinical change of the adrenal glands. It is concluded that the protective effect of oxidized starch sulfate and sulfate of reduced product of oxidized starch on ulceration of Shay rat is due to their inhibition of pepsin activity and ulceration of Shay rat is regarded as being caused mainly by proteolytic action of pepsin. Oxidized starch sulfate and sulfate of reduced product of oxidized starch of more than 1.5 moles/glucose unit sulfur content have enough protective effect on ulceration, even if they have low viscosity (0.009~0.001) and low anticoagulant activity. This result coincide with those of the inhibitory action on proteolysis by pepsin *in vitro*. It is known that polysaccharide sulfates have protective effect on ulceration,[1,2] but there was no report on the correlation between the protective effect and physicochemical properties (sulfur content and $\overline{DP}$) of polysaccharide sulfates. Attention should be paid to the undesirable effect upon hemorrhage from ulcer, because of their anticoagulant activity, but it was concluded that oxidized starch sulfates can be expected to have a new medicinal or prophylactic use against peptic ulceration, inhibiting directly the pepsin activity in gastric juice.

The author wishes to express his appreciation to Prof. Emeritus M. Ishidate, Prof. Y. Ito, and Prof. K. Takagi of the Faculty of Pharmaceutical Sciences, University of Tokyo, for their invaluable help and suggestion. He is also deeply grateful to Dr. S. Hayashi, Managing Director of this Company, and to Mr. G. Tatsui, Director of this Laboratory, for their permission to submit this report, and to Mr. H. Nakano and Mr. K. Iida for their technical assistances.

Summary

Sulfates of oxidized starch and its reduced products were investigated for their protective effect on experimental peptic ulceration.

1) Both oxidized starch sulfate and sulfate of reduced product of oxidized starch with various sulfur content and viscosity showed protective effect on ulceration of Shay rats when administered in 30 mg. doses intra-esophageally, and the protective effect is enhanced with increasing sulfur content, just the same as their inhibitory activity on proteolytic action *in vitro*.

2) Their protective effect on ulceration is closely related to the loss of pepsin activity in gastric juice. The decrease in pepsin activity is proportional to the decrease in ulceration rate. From the fact that they had no protective effect on ulceration by subcutaneous administration, their protective effect may be caused by their direct inhibition of pepsin activity in gastric juice.

3) They had no protective effect on Takagi's stress rats by intra-esophageal administration.

(Received February 16, 1961)