STRAIN DIFFERENCES IN ASPIRIN-INDUCED GASTRIC ULCERATION IN RATS

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Abstract—We found that there are strain differences in aspirin-induced ulceration in pylorus-ligated rats; the ulcer indices varied, from high to low, in the following order: Donryu > Sprague-Dawley > Wistar. Several experiments including analysis of gastric contents or ionic flux, determination of serum aspirin esterase activity, absorption of aspirin from the stomach, prothrombin time and hexosamine content in gastric mucosa and juice were performed to elucidate the origin of the differences. A significantly higher acid output in Donryu rats, and higher hexosamine content in the gastric mucosa of Wistar rats were noted. However, it appears unlikely that these factors only contribute to the marked strain difference in aspirin-induced ulcers. The possible different sensitivity of gastric mucosal cell itself to aspirin must be considered.

Aspirin induced gastric mucosal ulcers in several strains of rats, including Sprague-Dawley (1-3), Wistar (4, 5) or Donryu (6, 7) derived from the Wistar strain and which are used for laboratory experiments in Japan. We report herein different severity indices of aspirin ulcers in rats of three strains, and several factors which might contribute to the differences are discussed. To date there has apparently been no documentation concerning strain differences in aspirin-induced ulcers in rats.

MATERIALS AND METHODS

Male Donryu (Nihon Rat Co.), Sprague-Dawley (Clea Japan Inc.), and Wistar (Nihon Rat Co.) strain rats, weighing 180-200 g, were used. In all experiments described below, the animals were deprived of food but allowed free access to water for 24 hr before each procedure.

Induction of aspirin ulcers

Aspirin ulcers were produced by the standard method of Okabe et al. (6). Briefly, the pylorus of each strain of rat was ligated under ether anesthesia. Ten min later, aspirin 100 mg/kg suspended in 1% carboxymethylcellulose (CMC) solution was given orally in a volume of 0.5 ml/100 g of body weight. Seven hr after the aspirin administration, the animals were sacrificed and the stomach of each was removed and gastric contents were collected. Subsequently, the stomach was inflated by injection of 12 ml of 1% formalin and immersed in 1% formalin for 10 min. The stomach was then incised along the greater curvature and examined for the presence of gastric ulcers in the glandular portion. The length of each lesion (mm) was measured under the dissecting microscope (10×), summed

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and used as an ulcer index. The observer who measured the ulcers was unaware of which strain of rats had been tested. The gastric contents collected were centrifuged and analyzed for volume and acidity; the acidity was determined by titration of the gastric juice with 0.1 N NaOH to pH 7.0 by using the Autoburette (Radiometer). Acid output was expressed as $\mu\text{Eq/hr}$. The concentrations of Na^+ and K^+ ions were measured on a Hitachi flame-photometer; Na^+ and K^+ outputs were expressed as $\mu\text{Eq/hr}$. Pepsin activity was determined by Anson's method (8); pepsin output was expressed as mg tyrosine per hr. Ten samples of gastric juice were selected randomly for gastric content analysis. Control animals were given the same volume of 1% CMC solution alone.

Ionic flux through the gastric mucosa

Under ether anesthesia, the pylorus and cardia of each rat stomach were ligated and a subdiaphragmatic bilateral vagotomy was performed. A polyethylene tube was inserted into the forestomach through a small incision and secured with a ligature. After the abdomen was closed and the animals had recovered from anesthesia, the stomach was washed with saline. Eight ml of test solution (100 mM HCl and 54 mM NaCl), containing 40 mg/liter phenol red as a volume indicator, were instilled into the stomach through the fistula by a syringe. After gentle stirring, 2 ml of the solution were immediately sampled (I_1) and the end of the fistula was closed. One hr later, the residual solution was collected (F_1). One ml of aspirin suspension (100 mg/kg in CMC solution) was then injected together with 5 ml of test solution. As a control, CMC solution was used instead of aspirin. Thirty min later, the stomach was emptied and washed several times with saline, and again 8 ml of test solution was instilled and 2 ml was sampled (I_2). One hr after the latter procedure, the residual solution was collected (F_2). Each sample of I_1 , F_1 , I_2 and F_2 was analyzed for volume, concentration of phenol red, H^+ , Na^+ , K^+ and Cl^- . The concentration of phenol red was determined by the method of Hunt and Knox (9). Chloride concentration was determined by use of a chloride meter (Radiometer). The ion net flux (X_1) before the treatment of aspirin (or CMC solution alone) was calculated as follows: $X_1 = V_{F1} [X]_{F1} - V_{I1} [X]_{I1}$ where V_{F1} and V_{I1} are calculated from the measured volume and phenol red concentration, and $[X]$ is the concentration of each ion. The net flux after the drug treatment (X_2) was calculated the same way, and the change in mucosal permeability of an ion caused by aspirin (or CMC solution alone) was expressed as follows: $X = X_2 - X_1$.

Determination of aspirin acyl-hydrolase (aspirin esterase) activity

Determination of aspirin esterase activity was carried out according to the modified method of Menguy et al. (10). Blood was withdrawn from each rat via the vena cava and centrifuged for 30 min (4°C , 4,000 rpm). One ml of serum was mixed with 0.4 ml of 15 mM aspirin and 1.6 ml of 0.06 M Tris buffer (pH 7.0), and incubated for 1 hr at 37°C . The reaction was then stopped by adding 0.3 ml of 6 N HCl. The reaction mixture was shaken with 30 ml of ethylenedichloride for 5 min and centrifuged. Twenty ml of the organic phase was shaken with 10 ml of distilled water containing 0.25 ml of iron reagent (ferric nitrate 8.35 g in 500 ml of water) for 5 min. The optical density of the aqueous phase was measured

using a spectrophotometer (Hitachi) at 520 nm. Blanks were run through the procedure using Tris buffer instead of serum. Each sample was assayed in duplicate and the enzyme activity was expressed as μg salicylic acid/ml of serum/hr.

Measurement of salicylic acid and aspirin concentrations in blood

Six of the pylorus ligated rats were subjected to the determination of aspirin and salicylic acid concentrations 5, 15, 30, 60, 180 or 420 min after aspirin administration. Blood samples were collected from each rat and divided into 2 specimens of 3 ml each. One specimen was subjected to the measurement for total salicylic acid in blood and the other for aspirin. Immediately after the sampling, the blood of aspirin treated rats was shaken with 0.5 ml of 6 N HCl and heated at 100°C for 10 min (11). After hydrolysis, the total amount of salicylic acid in blood was extracted with ethylenedichloride and determined in the manner described above. A standard curve was obtained from the same procedure using blood of an intact rat containing a known amount of salicylic acid. Aspirin in blood was extracted directly by the method of Cotty and Ederma (12). Immediately after the sampling, the blood was mixed with 0.5 ml of 6 N HCl and shaken with 30 ml of ethylene dichloride for 5 min. After removing the blood layer by filtration, the organic phase was transferred to another tube. Then 0.1 ml of 40% ceric ammonium nitrate and 5.9 ml of water were added and the mixture was shaken for 1 hr. The aqueous layer was discarded and 20 ml of the organic phase was shaken with 4 ml of 1% NaHCO_3 for 10 min. Two ml of the aqueous layer was heated at 100°C for 1 hr. The concentration of salicylic acid was measured by spectrophotofluorometry in 10 N NaOH (Ex. 295 nm; Em. 405 nm uncorrected).

Prothrombin time

Quick's one stage prothrombin time test was performed in 12.5% serum using Bactothromboplastin (Difco) (13). The blood samples were obtained from pylorus-ligated rats which had been given either 100 mg aspirin/kg or the CMC solution 7 hr before the test. A 0.2 ml solution of 0.1 M sodium oxalate was added to 1.8 ml of the blood sample, and the preparation centrifuged (2,000 rpm, for 10 min). Serum (0.1 ml) was diluted by adding 0.7 ml of saline, and the prothrombin time was determined in this preparation according to the standard methods. These tests were done in triplicate.

Hexosamine content in gastric mucosa and juice

Both gastric mucosa and juice were obtained from pylorus-ligated rats that had been given aspirin or the CMC solution 7 hr before the sampling. After the gastric contents were collected, the rumen of each stomach was discarded and the glandular portion was placed in absolute ethanol, acetone and ether in this order. After drying in vacuum, the gastric mucosa was weighed. Hexosamine content in the tissue or in the gastric contents was measured by the method of Masamune and Yoshizawa (14). Briefly, the mucosa with 1 ml of distilled water or 1 ml of gastric juice was hydrolysed with 1 ml of 8 N HCl in a boiling water bath for 9 hr. After cooling, the hydrolysate was neutralized with 1.5 ml of 4 N NaOH, with the aid of phenolphthalein, followed by immediate addition of 0.3 N HCl until it became acidic to BTB. The hydrolysate was then made up to 10 ml with water and

filtered. Two ml of solution was heated in a boiling water bath for 20 min with 1 ml of acetylacetone reagent (1 ml of acetylacetone in 50 ml of 0.7 N Na_2CO_3). After cooling, the mixture was diluted with 6 ml of absolute ethanol and heated in a water bath (65–70°C) for 10 min with 1 ml of Ehrlich reagent (0.8 g of dimethylaminobenzaldehyde in a mixture of 30 ml of conc. HCl and 30 ml of absolute ethanol), and cooled in water prior to measurement at the wave length of 537 nm. Hexosamine content in the tissue was expressed as $\mu\text{g/g}$ of dry mucosa weight, and in the gastric juice, the value was expressed as $\mu\text{g/ml}$.

All values obtained were statistically analyzed using Student's *t*-test.

RESULTS

Aspirin ulcers

Ulcer indices of each strain of rats are shown in Table 1. Donryu strain rats showed a significantly higher ulcer index as compared with Sprague-Dawley or Wistar rats. The ulcer index of Sprague-Dawley rats was significantly higher than that of Wistar rats.

Gastric secretion

Table 2 shows the results of analysis of the gastric juice in pylorus-ligated rats given aspirin or 1% CMC solution. In the group of the animals given 1% CMC solution alone, the volume of gastric juice and acid output in Donryu rats was significantly higher than in the other strains. In the aspirin treated animals a significant reduction in acid output was confirmed in every strain and such was almost to the same degree. While an increment of Na^+ output was observed in every strain, the degree of change was not equal. The pepsin output in Donryu rats was found to be significantly higher than of Wistar rats.

Ionic fluxes in gastric juice

As shown in Table 3, aspirin caused a significant reduction of H^+ ion output and increment of Na^+ output. The degree of ion fluxes in rats used was almost the same in the three strains. The changes of K^+ and Cl^- ions were small in all three strains of rats.

Aspirin esterase activity

Aspirin esterase activity in Donryu rats was significantly higher than that of Sprague-Dawley or Wistar rats (Table 4).

TABLE 1. Aspirin ulcers in Donryu, Sprague-Dawley or Wistar strain rats

Group No.	Strain	No. of rats	Ulcer index (mm) mean $\pm$ s.e.
1	Donryu	40	43.7 $\pm$ 3.4
2	Sprague-Dawley	40	22.7 $\pm$ 2.9
3	Wistar	40	15.4 $\pm$ 2.4
	P		1:2 < 0.001
			1:3 < 0.001
			2:3 < 0.001

Aspirin at 100 mg/kg was given orally 10 min after pylorus ligation. The animals were sacrificed 7 hr after pylorus ligation.

TABLE 2. Effects of aspirin on gastric secretion in Donryu, Sprague-Dawley or Wistar rats with pylorus ligation

Group No.	Strain	Treatment	No. of rats	Gastric contents							Pepsin output (mg/jr)h	% change	
				Volume (ml)	% change	Acid output (μ Eq/hr)	% change	Na ⁺ output (μ Eq/hr)	% change	K ⁺ output (μ Eq/hr)			% change
1	Donryu	Control	10	13.9 $\pm$ 0.5		214.1 $\pm$ 9.9		75.9 $\pm$ 3.6		14.2 $\pm$ 1.1		42.1 $\pm$ 2.0	
2		Aspirin	10	11.9 $\pm$ 0.5	-14.4	115.7 $\pm$ 13.2	-46.0	135.1 $\pm$ 13.2	+78.0	11.4 $\pm$ 0.5	-19.7	40.9 $\pm$ 1.9	-2.8
3	Sprague-Dawley	Control	10	10.9 $\pm$ 0.8		156.4 $\pm$ 13.5		51.1 $\pm$ 5.5		10.7 $\pm$ 2.0		38.1 $\pm$ 2.3	
4		Aspirin	10	11.3 $\pm$ 0.9	+3.7	80.3 $\pm$ 13.9	-48.7	123.9 $\pm$ 11.9	-142.5	11.8 $\pm$ 1.1	+10.3	45.8 $\pm$ 2.8	+20.2
5	Wistar	Control	10	10.3 $\pm$ 0.6		157.6 $\pm$ 3.1		75.7 $\pm$ 5.4		13.6 $\pm$ 0.9		34.7 $\pm$ 1.5	
6		Aspirin	10	10.0 $\pm$ 0.6	-2.9	92.2 $\pm$ 7.2	-41.5	104.2 $\pm$ 7.0	-37.7	14.6 $\pm$ 0.9	+7.4	33.6 $\pm$ 1.8	-3.2
				1:2	<0.05	1:2	<0.001	1:2	<0.001	1:2	NS	1:2	NS
				1:3	<0.01	1:3	<0.01	1:3	NS	1:3	NS	1:3	NS
				1:5	<0.001	1:5	<0.001	1:5	NS	1:5	NS	1:5	<0.001
P				3:4	NS	3:4	<0.001	3:4	<0.01	3:4	NS	3:4	<0.05
				5:6	NS	5:6	<0.001	5:6	<0.01	5:6	NS	5:6	NS
				3:5	NS	3:5	NS	3:5	NS	3:5	NS	3:5	NS

All values represent mean $\pm$ s.e. The animals were sacrificed 7 hr after pylorus ligation. Aspirin at 100 mg/kg was given orally immediately after pylorus ligation. NS = Non-significant at the level of $P=0.05$.

TABLE 3. Effects of aspirin on ion flux in Donryu, Sprague-Dawley or Wistar strain rat stomach

Group No.	Strain	Treatment	No. of rats	Net changes of ion flux (μ Eq/hr)			
				H ⁺	Na ⁺	K ⁺	Cl ⁻
1	Donryu	Control	8	24.4 $\pm$ 6.3	27.1 $\pm$ 16.1	0.4 $\pm$ 0.8	55.6 $\pm$ 12.8
2		Aspirin	8	-58.6 $\pm$ 15.7	54.6 $\pm$ 8.4	2.3 $\pm$ 0.9	9.3 $\pm$ 15.4
3	Sprague-Dawley	Control	8	9.8 $\pm$ 15.7	-21.0 $\pm$ 15.2	-0.7 $\pm$ 0.6	-18.9 $\pm$ 7.3
4		Aspirin	8	-61.1 $\pm$ 16.9	88.2 $\pm$ 11.5	4.5 $\pm$ 1.0	- 6.3 $\pm$ 27.1
5	Wistar	Control	8	9.8 $\pm$ 4.8	2.5 $\pm$ 17.1	-0.2 $\pm$ 1.0	0.5 $\pm$ 29.6
6		Aspirin	8	-60.1 $\pm$ 8.9	39.5 $\pm$ 11.5	2.5 $\pm$ 0.7	-4.8 $\pm$ 16.5
P				1:2 < 0.001	1:2 NS	1:2 NS	1:2 < 0.05
				3:4 < 0.001	3:4 < 0.001	3:4 < 0.001	3:4 NS
				5:6 < 0.001	5:6 NS	5:6 NS	5:6 NS

All values represent mean $\pm$ s.e.

TABLE 4. Serum aspirin esterase activity in Donryu, Sprague-Dawley or Wistar strain rats

Group No.	Strain	No. of rats	Aspirin esterase activity (μ g salicylic acid/serum ml/hr) mean $\pm$ s.e.
1	Donryu	10	178 $\pm$ 10
2	Sprague-Dawley	10	136 $\pm$ 5
3	Wistar	10	140 $\pm$ 9
		P	1:2 < 0.001
			1:3 < 0.05
			2:3 N.S.

FIG. 1. Blood level of aspirin (ASA) and salicylic acid (SA) in Donryu, Sprague-Dawley or Wistar strain rats given 100 mg/kg of aspirin orally immediately after pylorus ligation. Each group included six rats of each strain.

Aspirin and salicylic acid in blood

Aspirin in the blood reached a peak value within 10 to 15 min after oral administration, after which the concentration rapidly decreased (Fig. 1). Changes in aspirin concentration were almost the same among the three strains. The level of salicylic acid reached a plateau within 30 to 60 min, and this level was maintained for 7 hr.

Prothrombin time

Prothrombin time in rats treated with 1% CMC solution alone was not significantly different among the three strains (Table 5). Aspirin significantly prolonged the prothrombin time in Sprague-Dawley rats only.

Hexosamine content

As can be seen in Table 6, the hexosamine content in gastric juice from Donryu, Sprague-Dawley and Wistar rats given 1% CMC solution alone did not significantly differ among the strains. Wistar rats, however, showed a significantly higher content of hexosamine

TABLE 5. Effect of aspirin on the prothrombin time in Donryu, Sprague-Dawley or Wistar rats

Strain	Treatment	No. of rats	Prothrombin time mean $\pm$ s.e.
Donryu	Control	6	38.4 $\pm$ 1.0
	Aspirin	6	45.6 $\pm$ 3.2
Sprague-Dawley	Control	6	39.8 $\pm$ 1.0
	Aspirin	6	62.4 $\pm$ 6.5*
Wistar	Control	6	42.8 $\pm$ 1.8
	Aspirin	6	53.2 $\pm$ 7.2

Aspirin at 100 mg/kg was given orally immediately after pylorus ligation. Prothrombin time was measured 7 hr after pylorus ligation. * $P < 0.05$.

TABLE 6. Hexosamine content in gastric mucosa or gastric juice in Donryu, Sprague-Dawley or Wistar strain rats

Group No.	Strain	No. of rats	Hexosamine content in the stomach ($\mu\text{g/g}$ mucosa or $\mu\text{g/ml}$ juice) mean $\pm$ s.e.		
1	Donryu	6	Mucosa	Control	1549 $\pm$ 15
2		6		Aspirin	1313 $\pm$ 37
3		6	Juice	Control	4104 $\pm$ 294
4		6		Aspirin	4641 $\pm$ 294
5	Sprague-Dawley	4	Mucosa	Control	1527 $\pm$ 58
6		4		Aspirin	1461 $\pm$ 67
7		5	Juice	Control	3644 $\pm$ 141
8		5		Aspirin	6032 $\pm$ 397
9	Wistar	5	Mucosa	Control	1771 $\pm$ 62
10		5		Aspirin	1388 $\pm$ 67
11		4	Juice	Control	3786 $\pm$ 413
12		4		Aspirin	5016 $\pm$ 387
P			1:2 <0.001	3:4 NS	5:6 NS
			7:8 <0.001	9:10 <0.05	11:12 NS
			1:5 NS	1:9 <0.01	5:9 <0.05

in gastric mucosa as compared with other strains. Hexosamine contents in gastric mucosa in the three strains tended to decrease with aspirin ingestion, while increase in the content in the gastric juice was noted.

DISCUSSION

The present studies indicate that there is a strain difference in susceptibility to aspirin ulceration in rats. The stomach of Donryu strain rats was found to be the most sensitive to aspirin, compared to Sprague-Dawley and Wistar strains. Sprague-Dawley rats were significantly more sensitive to the agent than Wistar rats. Various experiments were carried out to elucidate the contributing factors for these differences from physiological and pharmacological points of view.

It is widely accepted that a certain amount of gastric acid is a necessary factor for the

development of aspirin ulcers (15, 16). Thus, it was postulated that Donryu rats might secrete a much greater quantity of acid than Sprague-Dawley or Wistar rats. In fact, gastric analysis demonstrated that Donryu rats showed a significantly higher gastric acid output than Sprague-Dawley or Wistar rats. Therefore, there is the possibility that excessive acid might contribute to the high incidence of aspirin ulcers in Donryu rats. However, there was no difference in acid output between Sprague-Dawley and Wistar rats despite differences in ulcer indices of these groups.

It has been proposed that aspirin induces gastric ulcers by disrupting the gastric mucosal barrier resulting in the back diffusion of acid (17). The administration of aspirin produced a significant decrease of gastric acid output in the gastric juice, almost to the same degree in the three strains, and increment of Na^+ ion output in different degrees. These findings were also confirmed in the experiment done in the denervated rat stomach; ionic changes in response to aspirin were almost equal in Donryu, Sprague-Dawley or Wistar rats. These data indicate that the gastric mucosal barrier is much the same among the three strains.

Menguy et al. (10, 18) reported that aspirin esterase activity in human blood was lower in females and cirrhotic patients than in healthy males. Females and cirrhotic patients are known to be much more sensitive to aspirin induced bleeding or ulceration (19, 20). Therefore, they suggested that the low activity of aspirin esterase might contribute to the ulcerogenicity of the drug. Our present investigations showed that Donryu rats had a significantly higher activity of aspirin esterase than Sprague-Dawley or Wistar rats. Moreover, the degree of absorption and fate of aspirin from the rat stomach was almost identical among these three strains. Thus, these data show that aspirin esterase did not play an important role in the strain difference observed in aspirin ulcers in rats.

It was reported that chronically injected aspirin tends to prolong platelet aggregation (21). Our results showed that the prothrombin time of each strain of rats was almost equal. Only Sprague-Dawley rats indicated a prolongation of prothrombin time in response to aspirin.

Finally, hexosamine is one of the mucus components considered to play an important role in the maintenance of integrity of gastric mucosa (22). We observed that the hexosamine content in gastric mucosa of Donryu rats was significantly lower than that in Wistar rats, but such was not the case in the gastric juice. Here the difference was large, and such may not have contributed to the marked resistance of gastric mucosa of Wistar rats to aspirin. The administration of aspirin resulted in a reduction of hexosamine in the gastric mucosa and increase of the amine in the gastric juice, almost to the same degree in all three strains of rats.

It appears that the Donryu strain is the best of the three used herein for determining the high incidence of aspirin ulceration, for the study of ulcerogenicity of aspirin, and for the assay of possible antiulcer agents for aspirin studies.

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