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An Approach to the Evaluation of Oral Antihistaminic Preparations using Inhibitory Action of Histamine Skin Response in Dogs[1]

Takashi Hiraoka and Jun Serizawa

Product Development Laboratories, Sankyo Co., Ltd.[2]

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An approach to the evaluation of antihistaminic preparations were studied by quantitative measurement of the inhibitory action of histamine skin response in intact dogs. The size of the papule, which was produced by subsequent intracutaneous injection of histamine in the foreleg of dogs, was quantitatively measured without any intravenous injection of dye. The inhibition of histamine skin response was proportional to the increase of the dose of chlorpheniramine maleate and of clemastine fumarate which were given orally. The linear regression between the logarithmic dose and the tested area was highly significant in both cases. Clemastine was approximately five times more effective than chlorpheniramine when analyzed by the parallel line assay of the dose response relationship. The intensity and the duration of the inhibitory effect of clemastine tablets were compared with those of chlorpheniramine tablets following oral administration and it was found that the effects of the former was stronger and longer than the effects of the later. From these observations, it is concluded that this method seems to be useful for the evaluation of the effects of oral antihistaminic preparations during preclinical test.

The evaluation of the intensity and duration of action of oral pharmaceutical preparations has been usually studied by means of chemical determination of drug in blood. This approach is particularly valuable in case the drug level correlates well with the pharmacological activity, but is limited its application due to problems of analytical procedure arising from low drug levels. On the other hand, when a pharmacological action is quantitatively measureable directly with good accuracy in intact animals, this will be useful for the evaluation of many kind of preparations as a preclinical test.

The present report is concerned with an approach to quantitative evaluation of oral antihistaminic preparations by using inhibitory action of histamine skin response in intact dogs. This test has been used in clinical pharmacological evaluations of antihistaminics,[3] and yet little consideration has been given to quantitating it.

Material and Method

Histamine dihydrochloride dissolved in physiological saline, equivalent to 1 μg of histamine per ml, was freshly prepared from a stock solution containing 1 mg of histamine per ml.

Chlorpheniramine was a mixture of chlorpheniramine maleate and lactose (1: 24), and clemastine was a mixture of clemastine fumarate and lactose (1: 99), in gelatin capsule.

Chlorpheniramine tablets, containing 2 mg of chlorpheniramine maleate per tablet, and clemastine tablets, containing 1 mg of clemastine per tablet, were used as antihistaminic preparations.

Healthy mongrel dogs weighing 9.7 to 13.0 kg were used after fasting for about eighteen hr. A volume of 0.05 ml of histamine solution was intracutaneously injected in the foreleg of dogs where the hair had been cut well with an electric clipper. Injection site was selected at random on the skin surface of the foreleg, separating at least by 2 cm each other. Ten min after the injection, the length and breadth of the papule produced were measured with vernier calipers and the product of these 2 values was expressed as histamine response value. Before administration of antihistaminics the control value (R_0) of histamine response was measured, and after oral administration of antihistaminics, 0.05 ml histamine solution was injected at 1

1) A part of this report was presented at the 90th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, July 1970.

2) Location: *Hiromachi 1-chome, Shinagawa-ku, Tokyo.*

3) L. Joubert, Z. Gaut, and W.B. Abrams, *Clin. Pharmacol. Therap.*, **10**, 250 (1969).

or 2 hr intervals in a different site and then 10 min later response value (R_t) was measured. The inhibition rate was calculated as follow; inhibition rate (%) $= (1 - R_t/R_0) \times 100$. The inhibition rate was plotted against the time after administration of antihistaminics and the area under the inhibition rate curve was measured with a planimeter.

Result

Time Course of Histamine Response Value after Intracutaneous Injection of Histamine

The histamine response values were measured 5, 10, 20, 30 and 40 min after an intracutaneous injection of 0.05 ml histamine solution in 4 dogs. The papule appeared clearly 5 min after the injection and reached the highest value after 20 min. The outline of the papule was clearly seen after 10 min but was diffused 20 min later. Therefore in the following studies, the measurement of the response value was carried out 10 min after the histamine injection.

Relationship between Histamine Doses and Response Values

The response values were measured 10 min after the intracutaneous injection of 0.005, 0.05 and 0.5 μg histamine and 0.05 ml physiological saline on the foreleg of 6 dogs. The relationship between the logarithmic doses of histamine and the response values is presented in Fig. 1. The response value increased proportionally to the dose of histamine injected, and the linearity was observed between the logarithmic dose and the response value. The median dose 0.05 μg used in this preliminary experiment, was used also in the following studies.

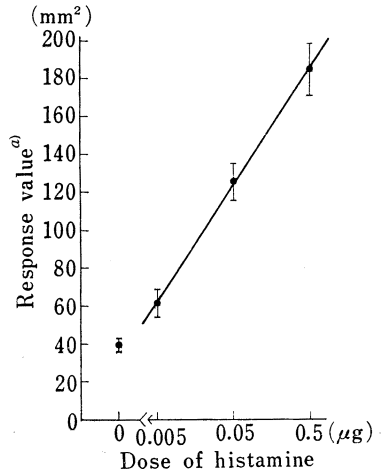

Fig. 1. Relationship between the Dose of Histamine Injected intracutaneously and the Response Value

a) Vertical lines indicate the standard error of the mean.

Variations in Response Value during the Course of Experiment

There were no significant variations in the size of the papules obtained by histamine injection during 11 hr of observation; the control response values (R_0) and the response values

Table I. Variations in Response Values during the Course of Experiment in Untreated Dogs

	Control	Time after control measurement (hr)					
		1	2	4	6	8	10
Response value (mm²)	154.9[a] ±6.8	151.8 ±4.9	154.1 ±5.6	153.8 ±3.6	156.8 ±4.1	152.3 ±3.4	152.4 ±4.8
Inhibition rate (%)	0	1.6[a] ±2.7	0.2 ±2.8	−0.5 ±5.8	−2.0 ±4.6	0.7 ±4.8	1.3 ±1.9

	Dog					
	A	B	C	D	E	F
Response value in individual dog (mm²)	147.8[b] ±3.3	159.9 ±2.2	165.6 ±2.3	149.3 ±2.2	144.2 ±5.2	155.6 ±3.8

a) mean±S.E. ($n=6$) *b*) mean±S.E. ($n=7$)

(R_t) were determined 1,2,4,6,8 and 10 hr after the control measurement in 6 dogs (Table I). The average values of 6 dogs at each given time varied little and the inhibition rates were kept within about two per cent. The average response of each dog during the experiment gave a marked difference. The data shown in Table I were subjected to statistical analysis. A significant difference in the histamine reaction was found among the dogs, whereas there was no significant changes in the size of the papules produced during 11 hr period. From these results, a crossover test was carried out in the following experiments after a resting period of at least one week.

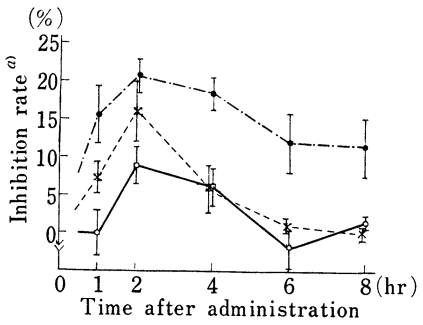

Fig. 2. Time Course of Inhibition Rate after Oral Administration of Chlorpheniramine in Three Doses

—○—: 6 mg/dog ($n=6$)
------×------: 12 mg/dog ($n=6$)
·—●·—·: 24 mg/dog ($n=6$)
a) vertical lines indicate the standard error of the mean

Dose-Response Relationship of the Inhibitory Effect of Antihistaminics

Fig. 2 and 3 show the time course of the inhibition rate of chlorpheniramine in doses of 6, 12 and 24 mg, and that of clemastine in doses of 1.5, 3 and 6 mg, which were obtained in crossover tests in 6 dogs. When compared with the results shown in Table I, it is clear that the histamine response values are inhibited at a maximum rate of 20% by both antihistaminics. The maximum inhibition was observed 2 hr after the administration of chlorpheniramine in the three doses tested. A peak of inhibition was not clearly noticeable in the three doses assays of clemastine because the inhibitory effect of clemastine could be observed for 8 hr. The inhibition rate became stronger and longer when the dose of either chlorpheniramine or clemastine was increased, thus indicating that there is a significant relationship between the doses and the areas under testing, which correspond to the intensity and duration of the action of drugs. As shown in Fig. 4, the linear regression between the logarithmic doses and the tested areas was observed unequivocally for the drugs tested.

In order to compare the activity of chlorpheniramine and clemastine, the data shown in Fig. 4 were statistically analyzed using the parallel line assay. As the parallelism of the

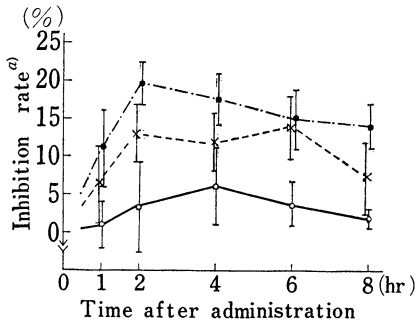

Fig. 3. Time Course of Inhibition Rate after Oral Administration of Clemastine in Three Doses

—○—: 1.5 mg/dog ($n=6$)
------×------: 3 mg/dog ($n=6$)
·—●·—·: 6 mg/dog ($n=6$)
a) Vertical lines indicate the standard error of the mean.

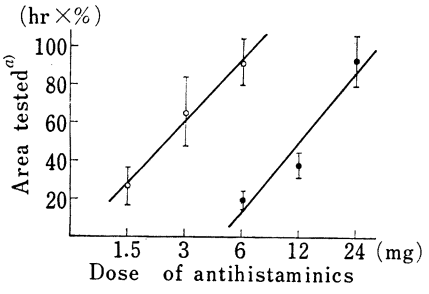

Fig. 4. Relationships between the Doses of Two Antihistaminics and the Area Tested as shown in Fig. 2 and 3

●: chlorpheniramine
○: clemastine
a) Vertical lines indicate the standard error of the mean.

two lines was significantly clear, it was concluded that clemastine was approximately five times more effective than chlorpheniramine.

Time Course of Inhibition Rate after Oral Administration of Chlorpheniramine and Clemastine Tablets

Fig. 5 shows the time course of inhibition rate after oral administration of 6 tablets of chlorpheniramine and clemastine. The maximal inhibition was found 2 hr after the administration of chlorpheniramine tablets and the inhibition rate returned to the control level after 6 hr. On the other hand, the inhibition rate 1 hr after the administration of clemastine tablets was higher than that of chlorpheniramine and the antihistaminic effect was maintained for 10 hr. When 16 mg of chlorpheniramine were given twice at 4 hr interval, its inhibitory effect was maintained for 10 hr; the same effect was obtained with a single dose of 6 mg of clemastine (Fig. 6). From these results, it is clear that the effect of clemastine tablets is approximately twice longer than that of chlorpheniramine tablets.

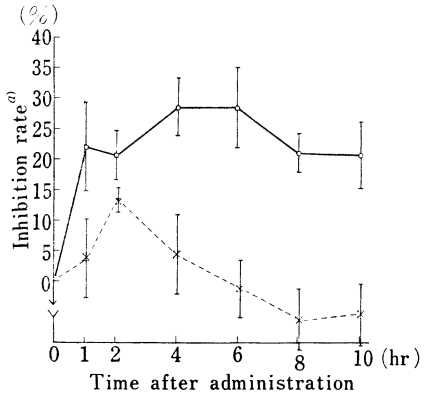

Fig. 5. Time Course of Inhibition Rate after a Single Oral Administration of Chlorpheniramine and Clemastine Tablet

---×---: chlorpheniramine 6 tab./dog ($n=6$)
—○—: clemastine 6 tab./dog ($n=6$)
a) Vertical lines indicate the standard error of the mean.

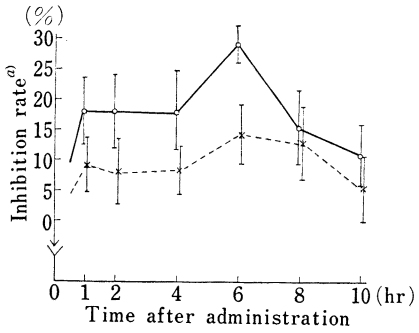

Fig. 6. Time Course of Inhibition Rate after Oral Administration of Two-fold Dose of Chlorpheniramine Tablet and a Single Dose of Clemastine Tablet

---×---: chlorpheniramine each 4 tab./dog administered at 0 and 4 hr ($n=6$)
—○—: clemastine 6 tab./dog ($n=6$)
a) Vertical lines indicate the standard error of the mean.

TABLE II. Variations in the Control Response Values Obtained from Six Dogs repeatedly Used for Nine Times

	Day								
	1	2	3	4	5	6	7	8	9
Control response value per day (mm^2)[a]	175.0 ±19.8	159.5 ±5.0	180.1 ±6.4	156.6 ±7.4	165.8 ±3.9	158.1 ±6.6	155.8 ±8.8	160.4 ±7.4	141.1 ±6.9

	Dog					
	A	B	C	D	E	F
Control response value in individual dog(mm^2)[b]	155.8 ±4.0	174.3 ±10.5	153.1 ±6.4	170.8 ±8.9	141.6 ±4.5	172.7 ±7.5

a) mean ±S.E. ($n=6$) *b*) mean ±S.E. ($n=9$)

Variations in the Control Response Value Obtained from 6 Dogs repeatedly Used

In the present studies, 6 dogs were repeatedly used 9 times with resting period of 1 or 2 weeks. The average control response value of 6 dogs in each experimental day and that of the individual dog during the course of this study are presented in Table II. By analysis of variance a significant difference was observed between dogs.

Discussion

The use of dogs or rabbits has been considered as experimental animals for the evaluation of oral dosage forms, such as tablets, capsules and so on. Dogs were selected in the present studies, since they were closer than rabbits to human in respect to the gastrointestinal absorption of drugs.[4,5] The inhibitory action of histamine skin response was used as a criterion of the pharmacological activity of the substances tested.[6] Using this method it was possible to observe continously the response of dogs which were not undergoing any anesthesia or restraining, thus inhibitory effects on the absorption of drugs from the gastrointestinal tract could be avoided.

When a histamine solution was intracutaneously injected in the forelegs of dogs, a papule appeared at the site of injection. The outline of the flare was irregular and its colour was not clearly differentiated from the colour of the dogs skin. In animal experiments, an histamine skin response has been usualy observed after the intravenous injection of a dye.[6a] In the present studies, the bluing response of the flare produced by histamine was also recorded after an intravenous injection of 1% solution of Evans blue. Immediatly after the injection of this dye, the skin of dogs was uniformly and lightly coloured. Only the flares showed marked bluing and the injected dye remained for at least 2 weeks in the dogs bodies. Therefore, in this experiment, the size of the papule produced was measured without injecting any dye.

Joubert, *et al.*[7] have described an histamine skin test in human subjects in which the size of the papules varies with the site of injection, the subject, the hour of the day and the day. In the present studies, significant differences were also observed between the dogs.

Using guinea pig ileum and anesthetized cats, Weidmann, *et al.*[8] reported that clemastine is approximately seven times more effective than chlorpheniramine; they found that the effect of clemastine lasted longer than that of chlorpheniramine, preventing acute histamine toxicity in guinea-pigs. Kerp, *et al.*[9] showed that the effect of clemastine is maintained for 10—12 hr after oral administration in human subjects. The results obtained in the present studies agree with their findings and it indicates that the approach is suitable for evaluating and comparing the intensity and the duration of effect of oral preparations with antihistaminic properties.

Chlorpheniramine and clemastine tablets used in the present studies were uncoated tablets which disintegrate within 10 min according to the disintegration test of the J. P. VIII. After oral administration of tritium labeled chlorpheniramine[10] or clemastine[8] to dogs,

4) W.L. Chou, S. Riegerman, and J.R. Amberg, *Chem. Pharm. Bull.* (Tokyo), **17**, 2170 (1969).
5) *a*) E. Rosen, T. Ellison, P. Tannebaum, S.M. Free, and A.P. Closley Jr., *J. Pharm. Sci.*, **56**, 365 (1967); *b*) W.A. Cressman and D. Summer, *ibid.*, **60**, 132 (1971); *c*) J. Serizawa, T. Hiraoka, and K. Sasahara, Abstracts of the 90th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, July, 1970, p. IV-38.
6) *a*) I.I.A. Tabachnick and F.E. Roth, "Animal and Clinical Pharmacological Techniques in Drug Evaluation," ed. by J.H. Nodine and P.E. Siegler, Year Book Medical Publishers, Inc., Chicago, 1964, p. 246; *b*) P.E. Siegler, *ibid.*, p. 253.
7) L. Joubert and G.B. Thomas, *J. Clin. Pharmacol.*, **10**, 165 (1970).
8) H. Weidmann, J. Grauwiler, R. Griffith, D. Römer, M. Taeschler, and K. Zehnder, *Boll. Chim. Farm.*, **106**, 467 (1967).
9) L. Kerp, H. Kasemir, and P.N. Tie, *Med. Welt.* (Stuttg.), **17**, 2794 (1968).
10) J.J. Kamm, C.R. Frerullo, D. Miller, and E.J.V. Loon, *Biochem. Pharmacol.*, **18**, 659 (1969).

the radioactivity in blood was detected for more than 24 hr. The difference between the retention time of labeled chlorpheniramine and clemastine obtained by Kamm, *et al.*[10] and by Weidmann, *et al.*[8] and the duration of effects of those two drugs observed in the present studies must be clarified in futher experiments in correlation with the metabolism of both drugs.

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