INHIBITORY ACTION OF SODIUM SALICYLATE ON THE GROWTH OF UPPER JAW AND TIBIA IN RATS

Shoji YAMADA

Department of Pharmacology, School of Dentistry, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113, Japan

Accepted November 4, 1976

Abstract—The inhibitory effect of sodium salicylate on the growing upper jaw and tibia was examined by the morphological measurement by radiography at different dose levels. The longitudinal growth of the tibia was greater than the sagittal growth of the upper jaw in young normal rats. The increment in length was approximately 10 mm a month in the former and 3 mm a month in the latter. Growth of these bones was reduced by successive subcutaneous injections of sodium salicylate, and the reduction of growth increased with increase of the dose administered. Sagittal growth of the upper jaw was much more sensitive to the action of salicylate, and was significantly reduced by the drug even at a dose of 100 mg/kg (P<0.01).

Many studies have been carried out on the action of salicylates on connective tissue metabolism in relation to anti-inflammatory action (1-4). On the other hand, there are comparatively few reports on the action of salicylate on calcified tissue in mammals, such as the bone and dentine which are types of connective tissue. Effects of a relatively large dose of aspirin on growing bones in rats was described by Mutch using radiography in 1934. He stated that aspirin broadened the calcified zone in the ossifying cartilage (5). Abbott and Harrison (6) reported a similar phenomenon. Cooper et al., using a tissue culture technique, demonstrated the inhibitory action of aspirin on collagen biosynthesis in bone (7). In these reports, the action of the salicylate was neither described in detail nor were any comments made on the mechanism of action of the salicylate on the growing bone in the living animal. On the other hand, Futami (8) reported that successive administration of a relatively large dose of sodium salicylate in the rat caused a significant reduction on the longitudinal growth of the tibia and suggested that the toxic action of the drug on the growing bone may be attributed to its inhibitory effect on physiological resorption or remodelling mechanism in the growing bone. However, there are apparently no reports concerning the dose-response relationship of the inhibitory action of salicylate on the growing bone in the living animal.

The purpose of the present study was to compare the effect of various dose levels of sodium salicylate on the growing upper jaw and tibia in the rat.

MATERIALS AND METHODS

Forty male Wistar strain rats, 30-31 days old and with a mean body weight of 80 g, were divided into the groups shown in Table 1. Sodium salicylate (special grade) was obtained from the Koso Chemical Co., Ltd., Tokyo, Japan. In order to determine the
bone growth during the experimental period of 30 days, rats in one group (untreated control, 30-31 days old) were decapitated under ether anesthesia at the beginning of the experiment, and measurements were carried out in the same way as in other groups. The sodium salicylate was so dissolved in 0.9 % NaCl solution that 5, 10 and 20 (w/v)% solutions were available. Three groups of rats (salicylate groups) were injected s.c. 15 times every two days with the different concentrations of sodium salicylate solution as stated above at a volume of 0.2 ml/100 g of body weight, namely, equivalent to 100, 200 and 400 mg/kg of body weight, respectively. The other one group of rats (control) was injected s.c. with the same volume of 0.9 % NaCl solution. Throughout the experiment, the animals were housed in groups of 4 in mesh, wire-bottomed cages and provided a standard diet (CE-2, Nippon Clea Co., Ltd., Japan) and tap water ad libitum. During the experimental period, the room temperature was kept at 20-22°C and the relative humidity at 37-57%. Two days after the last injection these animals were decapitated under ether anesthesia. The skulls and tibiae removed and freed of adhering muscle and soft tissue. The skulls were divided into halves at the midline with a single edge safety razor blade.

The right side of these skulls and tibiae were radiographed by using a Softex EMB-type apparatus (Softex Co., Ltd., Japan). The radiographs were taken right laterally for the skull and posterior-anteriorly for the tibia, respectively (Fig. 2-a and 3-a). Pictures were taken with 70 V, 30 mA and 60-80 sec using Fuji Softex Film-F.G. (Fuji Photo Film Co., Ltd., Japan) and developed for 5 min in Sofdol (Fuji Photo Film Co., Ltd., Japan) at 20°C. To avoid error in tracing, these radiograms were printed out on the photographic paper by the contact method. Measurements were carried out 5 times at every site on these papers using dial callipers with a minimum scale of 0.05 mm, and the average of these measurements was taken.

Landmarks employed in the measurement of the upper jaw were as follows (Fig. 2-b): 1) Point Ac; the alveolar crest on the concave (lingual) side of the incisor. 2) Point Mo; the most anterior point of the crown of the first molar. The distance between the two landmarks (Ac-Mo) was measured as an indicator to determine the growth of the upper jaw. The length (Ac-Mo) almost corresponds to the diastema which is the space between the first molar and the incisor.

As illustrated in Fig. 3-b, the distance between the proximal and distal epiphysis of the tibia was measured on the central axis; this indicates the length of the tibia.

RESULTS

The increase pattern in body weight in the control and salicylate groups is shown in Fig. 1. The control animals exhibited a typical growth pattern, and the animals injected with 100 mg/kg of sodium salicylate showed almost the same pattern as the control. Throughout the entire experimental period, the animals injected with 200 and 400 mg/kg of salicylate grew at a fairly slower rate than the control animals, and the reduction in body weight was proportional to the dose of the sodium salicylate administered.

The effect of salicylate on the growth of bone was examined using two different bones,
Fig. 1. Change in body weight of rats during the experimental period.

Fig. 2. (a) Enlarged radiogram of rat skull, right lateral projection. AC: The alveolar crest on the concave side of the incisor. Mo: The most anterior point of the crown of the first molar. S.I.: Premaxillomaxillary suture (sutura incisiva). (b) Schematic illustration of landmarks used for the measurement of the length of upper jaw. The distance between the point Ac and the point Mo was measured as an indicator to determine the growth of upper jaw.

Fig. 3. (a) Enlarged radiogram of rat tibia, post-anterior projection. B.S.: Bone shaft of tibia. D.D.: Distal diaphysis of tibia. F.: Fibula. P.D.: Proximal diaphysis of tibia. (b) Schematic illustration of the length of tibia.
TABLE 1. Effect of sodium salicylate on bone growth in rats

| Treatment          | No. of animals | Age in days | Diastema | Tibia   |
|--------------------|----------------|-------------|----------|---------|
| Untreated control  | 9              | 30          | 9.08±0.14 | 25.16±0.24 |
| Control            | 8              | 60          | 12.12±0.25 | 35.39±0.94 |
| Sodium salicylate  |                |             |          |         |
| 100 mg/kg          | 8              | 60          | 11.80±0.12 | 35.36±0.52 |
| 200 mg/kg          | 7              | 60          | 11.59±0.18 | 34.77±0.51 |
| 400 mg/kg          | 8              | 60          | 11.28±0.20 | 34.19±0.04 |

a. Animals were injected s.c. 15 times every two days for 30 days. b. Landmarks employed in measurement are shown in Fig. 2-b and 3-b. Values represent the mean±S.E. c. A group of untreated animals sacrificed at the beginning of the experiment. d. A group of animals injected s.c. with 0.9% NaCl solution. e. Significantly different from the control, P<0.01. f. Significantly different from the control, P<0.05. g. Not significant, P>0.05. h. In two tibiae, the proximal epiphysis was broken by the removal procedure and could not be measured.

The diastema (upper jaw) and the tibia (Fig. 2-b and 3-b). The results of each measurement are shown in Table 1. In the case of the tibia in the group injected with 400 mg/kg, two specimens were omitted because the proximal epiphysis was broken by the removal procedure. The linear growth increments at each measuring site in the normal rat for 30 days were calculated from the untreated control and control groups: The normal increment in the linear length of the diastema and tibia was approximately 3 and 10 mm, respectively. The length of the upper jaw decreased in proportion to the administered dose of salicylate, and by the t-test the difference between the control and all of the salicylate groups was found to be significant (P<0.01). The length of the tibia decreased in proportion to the dose of the salicylate, but there was only a significant difference (P<0.05) between the control and the group injected with 400 mg/kg of the drug.

The length of the upper jaw and tibia was reduced significantly in proportion to the administered doses of salicylate. In order to compare the effect of salicylate on these two growing sites, the growth reduction rate (°,0) of the length at each measuring site was calculated from Table 1 as follows:

\[
\text{Growth reduction rate} = \frac{\text{Reduction value}}{\text{Normal increment}} \times 100
\]

\[
\text{Normal increment} = \frac{\left( \frac{\text{Mean value of control}}{\text{untreated control}} \right) - \left( \frac{\text{Mean value of untreated control}}{\text{control}} \right)}{\text{Mean value of control} - \text{Mean value of untreated control}} \times 100
\]

\[
= \frac{\left( \frac{\text{Mean value of control}}{\text{salicylate group}} \right) - \left( \frac{\text{Mean value of untreated control}}{\text{control}} \right)}{\text{Mean value of control} - \text{Mean value of untreated control}} \times 100
\]
Fic. 4. Growth reduction rate of the length of tibia and diastema with successive administration of sodium salicylate (15 times every two days), calculated from Table 1, as described in the results.

The growth reduction rate means the intensity of the drug action at each dose level at the two target sites. Fig. 4 shows the relationship between the log dose of salicylate and the reduction rate of the upper jaw and tibia. The reduction rate at both sites increased with increase in the dose of salicylate. The dose of 100 mg/kg of salicylate caused an approximate 10% reduction in the growth of the upper jaw, whereas the growth of the tibia was reduced to the same degree by a dose of approximately 400 mg/kg. The results suggest that the growth of the upper jaw is much more sensitive to the effect of salicylate than that of tibia.

DISCUSSION

There are two main methods to determine the growth rate of hard tissue, such as the bone and dentine, one is histological and the other morphological. Examples of the former are the lead acetate method (9) and the vital staining method using dyes, such as alizarin red or tetracycline (10), etc. In these methods, however, the measurement of the growth rate is carried out on thin sections and cannot be done on the living animal. We found that the inhibitory effect of the drug on bone growth can be detected in situ by a rather simple, morphological measurement of the sagittal growth of the upper jaw even in the living rat. Although this method may be less accurate compared to histological measurements, nevertheless, such an application is considered appropriate for long-term experiments.

Rat skulls were radiographed after being divided into halves at the midline, in order that a clear radiographic image could be obtained. A radiographic sephalograrn was facilitated as the skull could be divided easily with a razor blade at the midline and the plane was useful to define the direction for radiography. The upper jaw of the rat consists of two bones on each side, premaxilla and maxilla (Fig. 5). On each side, these bones are joined
by the premaxillo-maxillary suture (sutura incisiva). At the posterior end of the maxilla, the maxilla is joined to the pterygoid by the pterygomaxillary suture, and it is also joined to the basisphenoid and the palatine by sutures. In the radiograms (Fig. 2-a), these sutures and the posterior margin of the hard palate are projected at the same spot on the film, and these images can be changed by slightly altering the projection angle. Therefore, the pterygomaxillary suture is unsuitable as a landmark. The growth of the upper jaw in the sagittal direction occurs mainly at the part of the premaxillo-maxillary suture and the pterygomaxillary suture. The expansion of the diastema was regarded as the growth of the upper jaw for the following reasons; both ends of the diastema show clear landmarks, and the premaxillo-maxillary suture which is one of the most important length-growth centers of the upper jaw is situated between both ends of the diastema.

The results presented in this study demonstrate clearly the following points with regard to growth of the upper jaw (diastema) and tibia: Growth in rats is inhibited with injection of sodium salicylate. The effect of the drug on the two sites is different in intensity; the growth of the upper jaw was reduced by approximately 10% by the successive administrations of 100 mg/kg salicylate, and approximately the same reduction was induced in the growth of the tibia by 400 mg/kg (Fig. 4). The difference in the intensity is attributed to the difference in the growing process at the two sites. Sicher and Weinmann (11) reported that the expansion of the diastema is achieved by the process of bone apposition and that it occurs directly at the part of the premaxillo-maxillary suture and by the distal drift of molars and apposition on the point Ac. They also reported that the upper molars of the young rat drift distally at a rate of about 60 to 80 microns a week. In the present study, the expansion rate of the diastema in the normal young rat was approximately 700 microns a week. Thus, the distal drift of the upper molars will be about one-tenth of the expansion rate of the diastema. Such implies that the expansion of the diastema is achieved, for the greater part,
by the appositional growth at the part of the premaxillo-maxillary suture. Ryo (12) reported that in young rabbits, the border between the premolars and the molars, that is the center of the six molars, is a fixed point against the maxilla during the upper jaw growth and that the growth increment of the diastema is achieved mostly by the appositional growth at the part of the premaxillo-maxillary suture. There are of course indirect factors such as the growth of the incisor, a masticatory apparatus (11), the growth of the nasal septum (13) and other adjacent bones that may affect expansion of the diastema. However, the net effect of these indirect factors on the expansion of the diastema is unclear.

On the other hand, the longitudinal growth of the tibia is achieved by the process of endochondral ossification. It is a most complicated process and the longitudinal growth of the tibia is not so simple as compared with the appositional growth pattern at the part of the premaxillo-maxillary suture (14–16). Such being the case, the effect of sodium salicylate on the growth of the tibia is not so detectable as in the case of the upper jaw. As indicated in Table 1, the mean reduction value of the length of the tibia in each group administered 100, 200 and 400 mg/kg of salicylate was 0.03, 0.62 and 1.20 mm, respectively, and that of the upper jaw was 0.32, 0.53 and 0.84 mm, respectively. The mean reduction value of the length of the tibia in the group on 200 mg/kg was similar to values in the upper jaw in groups on the same dose. However, the standard deviation of the length of the tibia was so large that a significant difference could not be observed in the length of the tibia of this group. The large value of the standard deviation may be attributed to the large variation of the longitudinal length of the tibia in the individual animal.

A significant decrease in the sagittal length of the upper jaw was found even in the group given 100 mg/kg of sodium salicylate s.c. every 48 hr for 30 days. When salicylate is used as an anti-rheumatic drug, adults are given a total daily dose of 5 to 8 g, in divided doses, and the best results are achieved when the plasma salicylate concentration is 25 to 35 mg% (17). The maximum plasma salicylate concentration in the rat after the intraperitoneal injection of 100 mg/kg of salicylate is approximately 30 mg%, and it decreases to below 20 mg% within 4 hours (18).

Acknowledgement: Gratitude is due to Professor H. Ogura for pertinent advice and discussion.

REFERENCES
1) Whitehouse, M.W.: Prog. Drug Res. 8, 301 (1965)
2) Smith, M.J.H.: The Salicylates. A Critical Bibliographic Review, Edited by Smith, M.J.H. and Smith, P.K., p. 49, Interscience Publishers, New York, London and Sydney (1966)
3) Paulus, H.E. and Whitehouse, M.W.: A. Rev. Pharmacol. 13, 107 (1973)
4) Ferrera, S.H. and Vanf, J.R.: A. Rev. Pharmacol. 14, 57 (1974)
5) Mutch, N.: J. Pharmacol. exp. Ther. 51, 112 (1934)
6) Abbott, D.D. and Harrison, J.W.E.: Fedn Proc. 24, 640 (1965)
7) Cooper, C.W., Doyt, S.B. and Talmage, R.V.: Proc. Soc. exp. Biol. Med. 117, 881 (1964)
8) Futami, K.: Bull. Tokyo Med. Dent. Univ. 20, 303 (1973)
9) Okada, M. and Mimura, T.: Japan. J. Med. Sci. IV. Pharmacol. 11, 166 (1938)
10) Frost, H.M.: Canad. J. Biochem. 41, 31 (1963)
11) Sicher, H. and Weinmann, J.P.: Oral Surg. 30, 109 (1944)
12) Ryo, S.: Ochanomizu Gakkai Zasshi, 1, 36 (1949) (in Japanese)
13) Ohyama, K.: Bull. Tokyo Med. Dent. Univ. 16, 157 (1969)
14) Vaughan, J.M.: The Physiology of Bone, p. 20, Clarendon Press, Oxford (1970)
15) Schenk, R., Merz, W.A., Mühlbauer, R., Russell, R.G.G. and Fleisch, H.: Calcif. Tiss. Res. 11, 196 (1973)
16) Lacroix, P.: The Biochemistry and Physiology of Bone, Edited by Bourne, G.H., Vol. 3, p. 119, Academic Press, New York and London (1971)
17) Woodbury, D.M. and Fingl, E.: The Pharmacological Basis of Therapeutics, Edited by Goodman, L.S. and Gilman, A., P. 338, MacMillan Publishing Co., Inc., New York (1975)
18) Nelson, E., Hanano, M. and Levy, G.: J. Pharmacol. exp. Ther. 153, 159 (1966)