Correlation between the analgesic and antipyretic effects of aspirin and salicylic acid concentration in rabbits

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Abstract: In order to clarify the correlation between the analgesic and antipyretic effects of aspirin and salicylic acid concentration, a jaw-opening reflex test and pyrogenic test were performed under the presence of aspirin, and the corresponding plasma concentration of salicylic acid was measured using rabbits. The analgesic effect of aspirin determined by jaw-opening reflex was significantly dependent on plasma level. The antipyretic effect of aspirin obtained by the pyrogenic test was also significantly dependent on plasma level. A closer correlation was demonstrated between the integral antipyretic effect and integral plasma level of salicylic acid.

Key words: Aspirin, analgesic effect, antipyretic effect, salicylic acid plasma concentration, correlation

Introduction

In order to administer drugs safely and efficiently, it is quite important to determine the concentration of drugs in the blood. Pharmacokinetics methods are generally used to monitor the drug concentration and presume the drug effects. Pharmacological activity including any untoward effect of a drug generally depends upon blood concentration, and the untoward effect appears when the blood concentration exceeds a certain threshold level (minimum toxic concentration). Drug monitoring has been performed of patients who suffer kidney or liver disease and who also differ to a large extent in the pharmacokinetics of a given drug from normals, as well as for drugs which have a therapeutic index that is quite narrow. Thus, blood concentrations of numerous drugs, including antiepileptics, antibiotics, antiarrhythmics, antiasthmatics, antineoplastics, and antidepressant drugs, have been measured routinely in order to establish an optimal therapeutic regimen for an individual patient at a given point in time. Recently, aspirin (Asp) has been used with the expectation that it would inhibit platelet aggregation at a low dosage, and has been subjected to therapeutic drug monitoring because a high blood level is necessary when antiinflammatory action is expected.

The present study was undertaken to clarify the correlation between the analgesic and antipyretic effects of Asp and salicylic acid (SA) plasma concentration. Thus, a jaw-opening reflex test and pyrogenic test were performed under the presence of Asp, and corresponding plasma concentrations of SA were also measured.

Materials and Methods

1. Animals

Japanese white strain male rabbits, weighing 2.5–3.5 kg, were purchased from Japan SLC, Inc., Japan, and kept under the following conditions: Room temperature of 23 ± 1 °C, relative humidity of 60 ± 10%, and lights on for 12 hours a day (7:00–19:00). The rabbits were kept on a commercial diet (RC4, Oriental Yeast Co., Japan) and filtered tap water ad libitum for the duration of the study. The body weight of the rabbits was 3.5–4.5 kg at the time of the experiment. Five
rabbits were used per group.

2. Drugs and chemicals

Asp was purchased from Wako Pure Chemicals Inc., Japan, and prepared as a fine powder (under 100 mesh). The Asp powder was suspended in 1% carboxymethylcellulose (CMC) solution to make 100mg/mL.

Lipopolysaccharide fraction of Escherichia coli was prepared in our laboratory from E. coli K-12.

3. Determination of analgesic effect

The analgesic effect was determined by the jaw-opening reflex test\(^3\)\(^,\)\(^4\). Thus, after fasting for 18 hours, the rabbits were anesthetized with 20mg/kg of pentobarbital sodium (Nembutal injection, Abbott, U.S.A.) through marginal auricular vein, and then kept on the back on a retainer. After the oral cavity was treated with a simple exclusion of moisture, the labial gingiva surrounding the mandibular central incisor was desquamated and the exposed alveolar bone was removed. A cavity reaching to the dental pulp of the incisor was made on the exposed root using a #1/2 round bar. A bipolar needle electrode (NM-250T, Nihon Kohden, Japan) was inserted into the cavity and the border of the cavity was sealed with dental utility wax. The electrode was connected to an isolator (SS-201J, Nihon Kohden, Japan) and then to an electric stimulator (SEN-7203, Nihon Kohden, Japan). The pulp was then stimulated five times with 50 V of potency, 500 ms of duration, and at intervals of 30 seconds to confirm the existence of a jaw-opening reflex. After the head of the rabbit was fixed to prevent moving, the tip of incisor of mandibular was connected to an isometric transducer (ME-4012, ME Commercial, Japan) and the jaw-opening reflex was recorded. After the steady state of the jaw-opening reflex was reached, the pulp was stimulated five times and the mean jaw-opening reflex was obtained as a normal reflex. Immediately after the withdrawal of 1 mL of blood from the heart by cardiopuncture, 100mg/kg of Asp (1 mL/kg in 1% CMC solution) was administered through a stomach tube. One per cent CMC solution, 1 mL/kg, was given to the control group. Determination of jaw-opening reflex and blood sampling were conducted 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 hours after administration of the Asp. Blood samples were placed in a heparinized test tube and then centrifuged at 11,000 × g for 30 seconds to obtain plasma for analysis of the Asp by high performance liquid chromatography (HPLC).

4. Determination of antipyretic effect

The antipyretic effect of Asp against febrility caused by administration of pyrogen was determined by measuring the rectal temperature of each rabbit\(^5\). Thus, after 18 hours of fasting, normal rectal temperature was measured by introducing a thermistor probe (PV, Nihon Kohden, Japan), connected to a thermistor thermometer (MGA III-219, Nihon Kohden, Japan), 7 cm deep into the rectum of each rabbit. The normal rectal temperature was obtained by measuring three times at 30 minute intervals. It was thus confirmed that the average variation in temperature was within 0.3°C. The generation of a fever by endotoxin of Escherichia coli, as a pyrogen, was then induced. Thus, 1 mL of lipopolysaccharide fraction of E. coli was administered through marginal auricular vein. The rectal temperature was measured three times at 30 minute intervals after administration of pyrogen, and a rise in temperature of 1.5°C was confirmed. After the third measurement, 1 mL of blood sample was obtained from heart by cardiopuncture, and then Asp (100 mg/kg) was administered through a stomach tube. The measurement of rectal temperature and blood sampling were conducted 0.5, 1.0, 1.5, 2.0, 3.0, and 4.0 hours after administration of the Asp. The blood samples were treated in the same manner as described above to obtain plasma for HPLC.

5. Determination of salicylic acid in plasma

The plasma concentration of SA was measured by the method reported by Rumble et al\(^6\). Briefly, 200µL of plasma was placed into a centrifuge tube (1.5mL), and then 200µL of 30% perchloic acid solution and 200µL of p-toluic acid solution (1 mg/mL MeOH) were mixed. The mixture was agitated for 2 minutes on a vortex mixer and then centrifuged for 3 minutes at 11,000 × g. The supernatant was filtered through a 0.22µm filter (UFC30GV00, Millipore, U.S.A.) by centrifugation. The filtrates (100µL) were diluted to 10-fold by addition of 50% ethanol and 20µL of the resulting fluid was injected into the HPLC for analysis. The analytical conditions are summarized in Table 1.
**Table 1** Chromatographic system and conditions

<table>
<thead>
<tr>
<th>Column</th>
<th>EYELA PACK TR-35-415 ODS 5 µm (Tokyo Rikakikai, Japan)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump</td>
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<tr>
<td>Column temperature</td>
<td>Ambient temperature</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>CH₃CN : MeOH : H₂O = 6 : 6 : 88</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0mL/min</td>
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<tr>
<td>UV detector</td>
<td>EYELA PLC-5 (Tokyo Rikakikai, Japan)</td>
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<tr>
<td>Integrator</td>
<td>C-R5A (Shimadzu, Japan)</td>
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<tr>
<td>Amount of infection</td>
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</tr>
</tbody>
</table>

**Fig. 1** Time-course analgesic effect of Asp by jaw-opening reflex in rabbits. Vertical axis is mean ratio against normal reflex (before Asp administration). Vertical bars indicate SD.

**Fig. 2** Time-course SA concentration in rabbit plasma during jaw-opening reflex test. Vertical bars indicate SD.

**Results**

1. **Analgesic effect**

Time-course changes of the jaw-opening reflex and the plasma concentration of SA are summarized in Fig. 1 and 2. The jaw-opening reflex decreased from 0.5 hours after administration of Asp to reach 49% of normal reflex at 2 hours after administration of Asp. The decrease in the jaw-opening reflex was negligible in the control group which was not given Asp. In the case of the plasma concentration of SA, tmax was 2 hours after administration of Asp. The correlation coefficient (r) between the relative decrement rate to 0 hours for the jaw-opening reflex (normal reflex) and the plasma concentration of SA was 0.98 (p = 0.0001), which indicated a close relationship (Fig. 3).

2. **Antipyretic effect**

The time-course change in the rectal temperature of the rabbits is shown in Fig. 4. The rectal temperature decreased from 0.5 hours after administration of Asp, and reached a temperature that was 1.12°C lower 4 hours after administration of Asp. In the case of the control group, two peaks in rectal temperature were found (Fig. 5). The plasma concentration of SA increased gradually to reach Cmax 2 hours after administration of Asp (Fig. 6). Fig. 7 indicates the time-course change of the true antipyretic effect, which was obtained by calculating the difference between the
Fig. 3  Correlation between SA concentration in plasma and reduction rate of jaw-opening reflex. Reduction rate is lowered percentage from normal reflex (before Asp administration).

Fig. 4 Time-course antipyretic effect of Asp on rectal temperature in pyrogenic test. Pyrogen was administered 1.5 hours before the administration of Asp. Vertical bars indicate SD.

Fig. 5 Time-course change of rectal temperature in rabbits. Pyrogen, extracted from Escherichia coli K-12, was administered iv at a dose of 1 mL/rabbit. Vertical bars indicate SD.

Fig. 6 Time-course SA concentration in plasma during pyrogenic test. Asp was given 1.5 hours after administration of pyrogen. Vertical bars indicate SD.

lower temperature of the test group and that of the control group from those at the time of Asp administration (1.5 hours after pyrogen administration). A correlation was found between the true antipyretic effect and plasma concentration of SA, $r = 0.820$, $p = 0.045$ (Fig. 8). Further, a closer correlation was found between the time-course integrated antipyretic effect and that of plasma concentration (AUC), $r = 0.997$, $p = 0.0001$ (Fig. 9).

Discussion

The hot plate method, thermal illumination method, Haffner's method, pressure loading method, electric stimulation method, and chemical stimulation method are commonly used to determine the analgesic effect7).

The jaw-opening reflex, produced primarily by contraction of the digastric muscle, is elicited when various sensory branches of the second and third divisions of the trigeminal nerve are stimulated electrically8).
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Fig. 7 Time-course change of antipyretic effect of Asp in rabbits. Antipyretic effect was calculated by the difference between change in rectal temperature in the test group and that in the control group (administered pyrogen only). Vertical bars indicate SD.

Fig. 8 Correlation between SA concentration in plasma and antipyretic effect.

Fig. 9 Correlation between AUC of SA and integrated antipyretic effect. AUC of SA is the area under the plasma concentration vs. time curve. Integrated antipyretic effect is the area under the antipyretic effect vs. time curve.

The degree of electric stimuli that stimulates the tooth pulp electrically is related to pain sensation9). It has also been reported that the onset of digastric electromyogram activity correlates with the appearance of $A\delta$ elevation in neurograms recorded for the maxillary nerve8, and that the percentage of the maximum amplitude of the component $A\alpha$, $A\delta$ and area covered by electromyogram responses from the digastric musc-

cle were related to multiples of the threshold intensity of the current pulses10).

A common pharmacological action of acidic non-steroidal anti-inflammatory drugs is the inhibition of cyclooxygenase resulting in the inhibition of prostaglandin (PG) synthesis11-13). PG, especially PGE, itself does not possess a strong algesic effect; however, it potentiates the algesic effect of bradykinin or histamine. Moreover, an algesic reaction occurs when PGE is administered simultaneously with bradykinin or histamine, or PGE is administered prior to the administration of bradykinin or histamine14).

Inoki et al.15) reported that a bradykinin-like substance was produced after electric stimulation of dental pulp. They also reported not only narcotic analgesics, but also Asp and aminopyrine were effective in inhibiting bradykinin production.

It might be possible that if the analgesic effect of Asp depends upon the inhibition of bradykinin release and an increase in the threshold of pain, then Asp possesses direct effect on dental pulp.

In man, Asp is rapidly hydrolyzed to SA6). Since the present data indicate the correlation between analgesic effect and SA plasma concentration, Asp/SA might effect the dental pulp directly.

Among pyrogens, exogenous and endogenous pyrogen, and PG are common16). Endotoxin of gram-negative bacteria, one of the most potent exogenous pyrogens, is a lipopolysaccharide consisting of a cell
membrane and is stable against heat. The pyrogenic reaction observed in the present study by administration of an extract from *Escherichia coli* gave two peaks in time-course rectal temperature, which clearly indicated the pyretic-pattern by bacterial endotoxin\(^\text{17}\). Thus, the first peak appeared 1.5 hours after administration of pyrogen, and the second peak at 3 hours. The first peak is considered to be a direct effect of pyrogen on the hypothalamus, and the second peak to be the effect of endogenous substance produced by leucocytes by the action of endotoxin\(^\text{17}\). The present findings suggest that the antipyretic effect of Asp is due to the inhibition of endogenous substance (the second peak). The authors could not confirm clearly the effect of Asp on the first peak. Interleukin-1 (IL-1) is one of the endogenous pyrogen\(^\text{16}\). It is generally accepted that IL-1 enhances intracephalic PG synthesis, which affects to thermoregulatory center to result in a higher set-point at the thermoregulatory center. Because Asp inhibits PGE synthesis, Asp may inhibit the upward displacement of the thermoregulatory set-point.

Stimulation by pyrogen differs very much from that by electric stimulation. The former effect is continuous while the later is transient. Thus, stimulation by pyrogen continues as long as the plasma concentration exceeds the minimum effective concentration level of SA in plasma. Although a correlation between antipyretic effect and the plasma concentration of SA was found, a closer correlation was obtained between the integral antipyretic effect and integral plasma concentration.

**Conclusion**

The correlation between analgesic or antipyretic effect of aspirin and salicylic acid plasma concentration was investigated to obtain the following results:

1) The analgesic effect of aspirin determined by the jaw-opening reflex was significantly dependent on salicylic acid plasma level.

2) The antipyretic effect of aspirin obtained by pyrogenic test was also significantly dependent on salicylic acid plasma level. Further, a closer correlation was demonstrated between the integral effect and the integral plasma level of salicylic acid.

These results indicate the presence of a close correlation between pharmacological activity and the plasma level of salicylic acid.

**References**

家兎におけるアスピリンの鎮痛作用および解熱作用とサリチル酸血漿中濃度の相関性

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藤井 彰

アスピリンの鎮痛効果および解熱効果とサリチル酸血漿中濃度との相関性を検討する目的で、家兎を用いて開口反射試験および発熱試験およびその時の血漿中濃度の測定を行った。開口反射試験を用いた鎮痛作用の場合、アスピリンの鎮痛効果はサリチル酸血漿中濃度に有意に依存していた。発熱性試験を用いた解熱作用の場合、アスピリンの解熱効果は血漿中濃度と有意な相関が認められたが、解熱効果曲線下面積と血漿中薬物濃度曲線下面積にさらに強い相関が認められた。

キーワード：アスピリン、鎮痛効果、解熱効果、サリチル酸血漿中濃度、相関性