ANTIPYRETIC MECHANISM OF INDOMETHACIN IN RABBITS

TAKAFUMI ITAMI, MAKOTO EMA AND SEIZABURO KANOH

National Institute of Hygienic Sciences, Osaka Branch, 1-1-43, Hoenzaka, Higashi-ku, Osaka, 540, Japan

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The mechanism of the antipyretic effect of indomethacin (IM) on fever induced by bacterial pyrogen (LPS, 0.2 μg/kg, i.v.), leukocytic pyrogen (LP, 2 ml/kg, i.v.) and 2,4-dinitrophenol (DNP, 20 mg/kg, i.m.) in male adult rabbits was studied. In plasma, the biological half lives of IM in normal and LPS-injected rabbits were estimated to be 24 and 21 min in the early phase and 72 and 51 min in the late phase, respectively. A potent antipyretic effect was observed with intravenous injection of IM in LPS- and LP-induced fevers, but not in DNP-induced fever. The antipyretic effect was also observed with intracisternal injection of indomethacin at doses of 0.025 and 0.013 mg/kg. The activity of endogenous pyrogen in serum after LP injection was not suppressed by the injection of IM (10 mg/kg, i.v.). The production of LP by leukocytes in vitro was not inhibited by IM (10 μg/ml). In our previous report, it was ascertained that the rectal temperature of normal rabbits remained unchanged after intravenous injection of IM.

These results suggest that indomethacin may inhibit only the pyretic processes in the central nervous system.

Keywords — indomethacin; antipyretic effect; bacterial pyrogen; leukocytic pyrogen; dinitrophenol

Indomethacin, 1-(p-chlorobenzoyl)-5-methoxy-2-methyindol-3-acetic acid (IM), one of the non-steroid anti-inflammatory agents, has been used as anti-inflammatory, analgesic and antipyretic agent.

Shen has described the chemical, pharmacological and biological studies performed with indomethacin including the uncoupling action of mitochondrial oxidative phosphorylation, inhibition of prostaglandin synthesis and stabilization of lysosomes. These activities are similar to that of acetylsalicylic acid and its metabolite, salicylic acid.

In a previous paper, we have reported the antipyretic effect of acetylsalicylic acid and salicylic acid on febrile rabbits induced by various pyrogens including bacterial endotoxin (LPS), 2,4-dinitrophenol (DNP) and leukocytic pyrogen (LP) which is a mediator of febrile response released from leukocyte and concluded that the mechanisms of the two antipyretics were different.

In this paper, we studied the antipyretic effect of IM on the febrile rabbit induced by various pyrogens and the results were compared with those of both acetylsalicylic acid and salicylic acid.

MATERIALS AND METHODS

1) Experimental Animals — Male Japanese white rabbits weighing 2.5 to 2.7 kg were used in all experiments.

2) Preparation of LPS and LP — LPS was extracted from Escherichia coli UKT-B strain by the hot-phenol method and was dissolved in saline. LP was prepared by the method of Kampschmidt et al.

3) Preparations of IM and DNP Solution — IM was dissolved in isotonic phosphate buffer (pH 7.4). DNP was dissolved in saline and adjusted to pH 7.4 with sodium bicarbonate. The solutions were filtered by Amicon PM-10 membranes before injection.

4) Measurement of Body Temperature — Rectal temperature was measured by a thermometer with thermistor detector (Iio Electric Co., Ltd).

5) Measurement of Pyrogenicity of Serum — Blood was collected by cardiac puncture at 2.5 h after LPS injection. Pyrogenicity of the serum obtained was measured by injection into the marginal ear veins of unanesthetized rabbits (2 ml/kg).

6) Withdrawing of Cerebrospinal Fluid (CSF) — CSF was withdrawn from the cisterna magna of rabbits without anesthesia by tuberculin syringe.

7) Determination of IM in Plasma — The plasma samples were assayed by modifying a
method described by Hucker et al. One half milliliter of plasma was pipetted into a glass stoppered centrifuge tube containing 1 ml of citrate buffer (0.5 M, pH 5) and 15 ml of heptane containing 3% isoamyl alcohol. The test tube was mechanically agitated vigorously for 15 min and then centrifuged. Ten milliliters of the heptane phase was pipetted into a second centrifuge tube containing 5 ml of 0.1 N NaOH and shaken mechanically for 15 min. The organic phase was separated by centrifugation and removed by aspiration. Two milliliters of aqueous phase was transferred to a quartz cell for immediate spectrofluorophotometric determination (Activation, 295 nm; fluorescence, 378 nm, uncorrected).

8) Statistical Evaluation—The obtained data were analyzed by the use of the Student's t-test.

RESULTS

1. Plasma Concentration of IM

Plasma concentration of IM was measured after intravenous injection of IM (10 mg/kg) in normal and febrile rabbits injected by LPS (0.2 μg/kg, i.v.). As shown in Fig. 1, the concentration of IM was 80 μg/ml immediately after injection of IM, but was less than 1 μg/ml after 4 h. The biological half lives of IM were 24 and 72 min in early and late phases, respectively, in normal rabbits. On the other hand, in febrile rabbits, the half lives in the two phases were 21 min in the early phase and 51 min in the late phase.

2. Effect of Intravenous Injection of IM on Fever Induced by LPS

The body temperature change was measured after an injection of IM in LPS-injected rabbits (Fig. 2). Significant antipyretic activity was observed with a dose of 2.5 mg/kg of IM injected at 0, 1 or 2 h after LPS (0.2 μg/kg, i.v.) and the activity was more significant when the dose was increased.

3. Effect of Intravenous Injection of IM on the Fever Induced by LP

The body temperature was measured after simultaneous injections of IM (5 mg/kg i.v.) and LP (2 ml/kg i.v.). As shown in Fig. 3, the febrile response induced by LP was significantly inhibited by IM.

4. Effect of Intravenous Injection of IM on the Fever Induced by DNP

Figure 4 shows that DNP (20 mg/kg, i.m.) evoked a fever and produced a maximum temperature rise of about 1.3 °C at 2 h after injection. When IM (5 mg/kg, i.v.) was given simultaneously with DNP injection, the DNP-induced febrile response was not significantly influenced by IM.

5. Effect of Intracisternal Injection (i.c.) of IM on the Fever Induced by LPS

IM (0.025 or 0.013 mg/kg) was injected into the cisterna magna of rabbits simultaneously with the intravenous injection of LPS (0.2 μg/kg). The dose dependent inhibition by IM given i.c. was observed on fever induced by LPS given i.v. (Fig. 5).

With the intracisternal injection, only 1/200 of the effective dose of IM in the intravenous study (Fig. 2) was needed for an antipyretic effect on the LPS-induced fever (Fig. 5).

To determine the concentration in CSF after IM administration (i.v.), CSF was withdrawn 1 h after IM injection (10 mg/kg, i.v.) in three rabbits and the CSF and plasma were assayed. The mean with standard error of the concentration of IM in the CSF was 0.3 ±0.2 μg/ml and in plasma was 8 ±0.9 μg/ml.

Antipyretic Mechanism of Indomethacin

Nishio et al. 5) reported that the maximum pyrogenic activity in serum was observed at 2–3 h after LPS injection in rabbits.

Blood was withdrawn from febrile rabbits at 2.5 h after LPS (0.2 μg/kg) was injected i.v. or from non-febrile rabbits at 2.5 h after injection of LPS (0.2 μg/kg, i.v.) with injections of IM simultaneously and 1 h after LPS injection. The serum (2 ml/kg) was then injected to other rabbits. The body temperature changes in recipients were measured (Fig. 6). As shown in Fig. 6, the pyrogenic activity was found in the plasma from not only the rabbits injected by LPS alone but also the rabbits treated by a combination of LPS and IM.

These results suggested that IM was unable to inhibit the production of EP in rabbits injected by LPS.

7. Effect of IM on the Production of LP by Leukocytes in Vitro

To clarify whether the production of LP by leukocytes could be inhibited by IM in vitro, leukocytes were incubated in the system of LP-preparation (Kampschmidt et al. 4) with or without IM (10 μg/ml). The concentration of IM was more than the plasma level when LPS-induced fever was inhibited significantly. The pyrogenicity of the supernatant fluid of the incubation medium was assayed. The results showed no significant differences in the pyrogenicity produced by the incubation mixtures containing or not containing IM. These results suggested that IM...
The mechanism of febrile response induced by LPS is not well understood, but it has been generally accepted that EP, including LP, are synthesized by the interaction of reticuloendothelial phagocytic cells with LPS and act as final mediators of the pyrogenic response within the brain. However, the possibility of direct action of LPS on central nervous system is not excluded.

Atkins\(^8\) reported that the biphasic fever induced by LPS might include two mechanisms, the early phase of fever might be induced by a direct action of LPS to the central nervous system and the later one might be by that of EP produced secondarily. The antipyretic effect of a drug might be revealed by the interference in one or a combination of several steps including the pyrogenic mechanism mentioned above.

As shown in Fig. 2, IM showed a potent antipyretic effect during the biphasic fever induced by LPS which may be due to: the inhibition on the production of EP or LP, the production of endogenous inhibitor against febrile response and the inhibition of febrile mechanism in the central nervous system. However, the pyrogenic activity in serum after LPS injection was not altered by IM injection (Fig. 6) and the production of LP-activity produced by leukocytes was also not inhibited by the presence of IM \emph{in vitro}.

These results indicated that IM did not alter the release of EP either \emph{in vivo} and \emph{in vitro}. Hoo \emph{et al.}\(^9\) demonstrated that the ability of the leukocytes to release LP was not altered by sodium acetylsalicylate \emph{in vitro}. Van Miert \emph{et al.}\(^{10}\) and Nishio \emph{et al.}\(^5\) reported no inhibitory activity by sodium salicylate and aminoptyrine on the LP-production or release of rabbit leukocytes \emph{in vitro}, respectively. But, Nishio \emph{et al.}\(^5\) found that aminoptyrine given \emph{i.v.} could reduce the EP activity in serum of rabbit injected with LPS.

These results indicated that IM had a different feature from aminoptyrine in the interaction with leukocytes of rabbit. On the other hand, the potent antipyretic effect was observed when IM was injected \emph{i.c.} (Fig. 5). These results suggested that the antipyretic effect of IM might be induced by the actions in central nervous system. These features of IM in the antipyretic activity on fever induced by various pyrogens were very similar to those of acetylsalicylic acid reported previously.\(^2\)

Acetylsalicylic acid-like drugs\(^{11}\) including
IM has been known as potent inhibitors of the synthesis of prostaglandin, which is accepted as a mediator in febrile response in the central nervous system.\(^{12}\) A relatively strong indication that prostaglandins mediate fever is provided by reports that those prostaglandin E\(_5\) series produce a rise in body temperature\(^{13,14}\) when injected into the hypothalamus of cats and rabbits. The increased levels of prostaglandin or prostaglandin-like materials were detected in cerebrospinal fluid when the body temperature was elevated by pyrogens.\(^{10}\) When the fever was suppressed by administration of antipyretics, the concentration of these materials also decreased. After an infusion of antibody to prostaglandin E\(_5\) into the preoptic/anterior hypothalamic region of a rabbit, an injection of LP caused little or no fever.\(^{16}\)

In our results, the antipyretic potency of IM given i.c. at 0.025 mg/kg of body weight was comparable to that of 0.35 mg/kg of sodium acetylsalicylate given by same route. IM was approximately 10 times more effective than sodium acetylsalicylate. Flower and Vane\(^{17}\) reported that the inhibitory potency of IM was about 10 times of sodium acetylsalicylate in brain prostaglandin synthetase.

These results suggested that the antipyretic effect of IM might be due to the inhibition of prostaglandin synthesis in the brain.

REFERENCES