PAIN RESPONSE OF RABBITS TO THE INTRAMUSCULAR INJECTION OF ANTIBIOTICS, WITH SPECIAL REFERENCE TO TIMOXICILLIN, A NEW PENICILLIN DERIVATIVE

Shiro TACHIKAWA and Akio TACHIBANA
Department of Pharmacology, Central Research Laboratories Yamanouchi Pharmaceutical Co., Ltd., Itabashi, Tokyo [174]
Received May 6, 1977

Summary......The quantitative analysis of nociceptive reaction produced by i.m injection of some algesic derivatives of penicillin and cephalosporin was carried out with rabbits. A half ml of the drug solution was injected into the antebrachial muscle of rabbits, and a characteristic behavior was scored by measuring the length of time during which the animals lifted a forelimb of the injected side. Since the response to 25% carbenicillin solution was reduced either by premedication with 1 or 3 mg morphine/kg i. v. in a dose dependent manner or by utilizing 0.5% lidocaine solution as a solvent for carbenicillin, the observed behavior may be regarded as the manifestation of pain experience in the animals. Carbenicillin, sulbenicillin and cephalothin produced the pronounced pain response, while cephalizin was relatively less noxious in rabbits. Timoxicillin, a new wide-spectrum penicillin, was the least painful among the sodium salt of antibiotics tested, Cephaloridine, which is not a sodium salt but a betaine form, showed no detectable pain response. The present results are consistent with many clinical experiences with respect to pain produced by these intramuscular remedies. The method described here seems useful for screening the compounds which are presumed to possess algesic activity.

An intramuscular injection of antibiotics frequently induced the local pain as one of unpleasant side effects. Indeed, a number of penicillin and cephalosporin derivatives often give rise to severe nociceptive reactions at the site of injection, and some of them are restricted in clinical use because of this unpleasant feature (Manten, 1972). Lidocaine solution, for instance, has been utilized as the solvent for the algesic antibiotics to relieve of the local pain. However, it was difficult to quantitatively evaluate the pain produced by i.m. injection of drugs in animals. Although the pseudaffective response to drugs have been evaluated by measuring the twitches of the skin of dogs (Hoppe et al., 1959) or by the electromyogram of the femur of rabbits (Naito et al., 1962), these procedures are rather complicated and not suitable for screening the new compounds which are

* The name timoxicillin will be proposed as its International Nonproprietary Name.
presumed to possess algesic side effect.

In the present study, we developed a new method for the quantitative analysis of pain produced by i.m. injection of drugs in rabbits, and the pain response to timoxicillin, a new semi-synthetic penicillin derivative with a broad antibacterial spectrum* (Tachibana et al., 1975) was compared with that of other well-known β-lactam antibiotics such as carbenicillin, sulbenicillin, cephalothin, cephaloridine and cephalozolin. Chemical structure of timoxicillin is as follows:

METHODS AND MATERIALS

Male albino rabbits weighing from 2.5 to 3.5 kg were used. Test substances were dissolved in distilled water or 0.5% lidocaine solution, and injected into the antebraclial muscle of rabbits in a volume of 0.5 ml. In some cases morphine was premixed through the ear vein. The rabbits were allowed to move freely on the floor (about 2×2 m) and their behavior was observed. The intensity of pain response to the injection was assessed according to the following scales:

Score 0: No behavioral change
1: A forelimb of the injected side was continuously lifted up and did not touch the floor within 1 min.
2: The behavior mentioned above continued for 1-3 min.
3: For 3-10 min.
4: For 10-30 min.
5: For 30 min or more.

The compounds used were timoxicillin (prepared in this laboratory), carbenicillin (Fujisawa Co., Gripenin®), sulbenicillin (Takeda Co., Lilacillin®), cephalothin (Torii Co., Ceporacin®), cephaloridine (Torii Co., Ceporan®), cephalozolin (Fujisawa Co., Cefamezin®), morphine hydrochloride (Tanabe Co.) and lidocaine hydrochloride (Fujisawa Co.).

All antibiotics used are in the form of the sodium salt except for cephaloridine which is in base form. Their concentrations are expressed in terms of W/W% and the lidocaine concentration in terms of W/V%. The pH of antibiotic solution was between 5.5 and 6.0.

Timoxicillin: Sodium salt of D(-)-α-[3-(4-oxo-4H-thiopyran)
carboxamido]-p-hydroxybenzylpenicillin

--- 274 ---
Pain response to Timoxicillin

RESULTS

A. Behavior of rabbits after i.m. injection of carbenicillin

Saline or 25% solution of carbenicillin in distilled water was injected into the antibrachial muscle of rabbits in a volume of 0.5 ml, and the behavior was observed. As seen in Fig. 1A, no behavioral change could be detected by saline injection, whereas an injection of 25% carbenicillin resulted in a characteristic behavior. The forelimb of the injected side was lifted up soon after the injection and this response lasted for several min.

Some of animals given carbenicillin i.m. jumped up in the sudden, stooped or hobbled about keeping their forelimb up (Fig. 1B). When the site of injection was pressed strongly with fingers of an observer, neither additional response such as screaming nor struggling to escape was elicited.

B. Effect of morphine and lidocaine on the behavioral response to the i.m. injection of carbenicillin

1. Effect of morphine

Mean scores were 2.9 for 13 animals which were intramuscularly given 25% carbenicillin solution. Morphine hydrochloride, 1 or 3 mg/kg i.v., was administered through the ear vein of rabbits, 5, 30 or 60 min prior to the i.m. injection of 25% carbenicillin. As shown in Fig. 2, morphine obviously reduced the scores of behavioral response to carbenicillin. The effect of morphine was dose-dependent and attained to the peak about 30 min after i.v. injection (Fig. 2). The spontaneous movement of rabbits was suppressed soon after morphine application and the degree of sedation augmented with time. However, the motor coordination was not significantly impaired throughout the experiment. This was ascertained by observing the response to sonic (clapping) or mechanical stimula-

![Saline](image1.png) ![Carbenicillin](image2.png)

Fig. 1. The body posture of rabbits 1 min after i.m. injection of saline (A) and 25% carbenicillin in distilled water (B). The solution was applied in a volume of 0.5 ml into the left antibrachial muscle of the rabbits. (A) No influence caused by the injection was observed. (B) The left forelimb was lifted up, and the body posture change was consistently observed for several min.
Shiro TACHIKAWA and Akio TACHIBANA

![Graph showing the effect of morphine on the behavioral responses of rabbits](image)

**Fig. 2.** Effect of morphine on the behavioral responses of rabbits to i.m. injection of carbenicillin (25% solution). The drug was dissolved in distilled water and applied in a volume of 0.5 ml into the antebrachial muscle. Morphine 1 mg/kg (••••••) or 3 mg/kg (×××××) was injected i.v. 5, 30 or 60 min before the experiments. Each point represents the mean ± S.E. Figures in parenthesis indicate numbers of animals used.

These results may suggest that morphine inhibited the behavioral response to carbenicillin probably because of its analgesic effect.

2. Effect of lidocaine

The characteristic behavior, i.e., lifting a forelimb of the injected side, after i.m. application of 25% carbenicillin solution in most cases lasted for 3 to 10 min (an example is shown in Fig. 3A). However, when carbenicillin in 0.5% lidocaine solution was injected to the animals, the response was somewhat less characteristic and its duration was obviously shortened when compared with the case in which carbenicillin was resolved in distilled water (Fig. 3B).

Intramuscular injection of timoxicillin also induced the characteristic response in rabbits, quite similar to that produced by carbenicillin. The intensity of response to timoxicillin was, however, much weaker than to carbenicillin, when the scores for equivalent doses of both drugs were compared (Fig. 4). Furthermore, a clear relationship was obtained between the dose of carbenicillin or of timoxicillin and the intensity of the behavioral change of rabbits. Lidocaine was also effective in reducing the scores of response to timoxicillin as in the case of carbenicillin (Fig. 4).

3. Behavioral response to i.m. injection of various antibiotics

A comparison was made for the intensities of responses to various antibiotics including penicillins and cephalosporins. The results were summarized in Table 1. Carbenicillin, sulbenicillin and cephalothin produced the pronounced nociceptive reactions,
Fig. 3. The time course of the body posture change (left forelimb) caused by 25% carbenicillin in distilled water (A) and in 0.5% lidocaine solution (B) applied in a volume of 0.5 ml into the left antebrachial muscle of the rabbits. (A) Change in position of the left forelimb remained even 10 min after the injection. (B) Recovery of normal body posture was observed by 5 min after the injection.

whereas cephazolin seemed less noxious. Timoxicillin was proved to be the least affective among the sodium salt of antibiotics tested. Of particular interest is that cephaloridine, which is not a sodium salt but a betaine form, showed a less affective response than five other antibiotics.
Shiro TACHIKAWA and Akio TACHIBANA

![Figure 4](image)

Fig. 4. Effect of lidocaine on the pain responses of rabbits to i.m. injection of timoxicillin and carbenicillin. The drugs were dissolved in distilled water (open columns) or in 0.5% lidocaine solution (striped columns) and were applied in a volume of 0.5 ml into the antebibrachial muscle. The vertical bars and figures in parenthesis indicate standard errors of mean and numbers of animals used, respectively.

Table 1. Scores of the behavioral responses to i.m. injection of timoxicillin and other antibiotics in rabbits.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Concentration (%)</th>
<th>No. of animals</th>
<th>Score (4:1; 3:0; 2:1; 1:2; 0:3)</th>
<th>Mean ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timoxicillin</td>
<td>25.0</td>
<td>6</td>
<td>0 0 1 5 0</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>25.0</td>
<td>13</td>
<td>0 12 1 0 0</td>
<td>2.9 ± 0.1</td>
</tr>
<tr>
<td>Sulbenicillin</td>
<td>25.0</td>
<td>8</td>
<td>2 4 1 0 1</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>Cephaloridine</td>
<td>25.0</td>
<td>6</td>
<td>0 0 2 4 0</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>20.0</td>
<td>8</td>
<td>0 6 1 1 0</td>
<td>2.6 ± 0.3</td>
</tr>
<tr>
<td>Cephalozolin</td>
<td>20.0</td>
<td>6</td>
<td>0 1 4 1 0</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td>Saline</td>
<td>–</td>
<td>10</td>
<td>0 0 0 0 10</td>
<td>0</td>
</tr>
</tbody>
</table>

DISCUSSION

Although the quantitative analysis of drug-induced pain has been carried out by several authors (Hoppe et al., 1959; Naito et al., 1962; Guzman et al., 1964; Taira et al., 1968), most of them aimed mainly at the field of the basic physiology or pharmacology in relation to the mechanisms of the pain production systems in the organisms. On the other hand, the symptoms following i.m. administration of many antibiotics comprise primarily pain at the site of injection (Hoigné, 1975). Therefore, in the process of screen-
ing new antibiotics using animals, it is necessary to obtain informations about not only the preferable but also noxious reactions induced locally. In the present time, however, practical screening methods to evaluate this kind of pain sensation of animals have not been well established. In this connection, we have studied the properties of pain responses produced by i.m. injection of some painful remedies using rabbits.

The i.m. injection of carbenicillin in concentrations which were generally used for the clinical trials revealed a characteristic behavioral response in rabbits; the forelimb of the injected side was lifted up. This response may be a manifestation of continuous dull pain but not of sharp and transient one, since the animals maintained this body posture for a few minutes and exhibited no additional response to extrinsic mechanical stimuli. These observations may be consistent with the clinical experiences about medication of this compound. The similar phenomena were also observed with other antibiotics except for cephaloridine.

This characteristic behavioral response to carbenicillin, i.e. lifting a forelimb of the injected side, was suppressed by morphine, 1 and 3 mg/kg i.v., without impairment of motor coordination of the rabbits, suggesting an exclusive participation of the analgesic activity of this narcotic drug. Furthermore, effect of lidocaine, a local anesthetic agent, on the pain responses to the antibiotics was investigated; the injection of carbenicillin or timoxicillin resolved in 0.5% lidocaine solution resulted in only a slight behavioral response when compared with the cases in which these antibiotics were resolved in distilled water. This result also coincides with the clinical observations that the local pain of antibiotics could be controlled with use of 0.5% or 3% solution of lidocaine (Cahn and Levy, 1972; Maurice et al., 1973).

The pain responses to many antibiotics available for the clinical use were compared each other. Cephaloridine is the only compound among the antibiotics tested that is not a sodium salt but a betaine form, and causes less pain than cephalothin (Manten, 1972) on i.m. administration to humans. In this study, cephaloridine produced slight behavioral response in rabbits and the intensity was less than the other five antibiotics tested. The order of the intensity of pain response to the sodium salt of the five antibiotics was as follows; carbenicillin > sulbenicillin > cephalothin > cephalazolin > timoxicillin.

Recently, Saito (1976) have found that the i.m. injection of 25% timoxicillin solution which did not contain any local anesthetic drug could be well tolerated by healthy volunteers, who complained only of a slight and very transient local pain. This evidence seems to support the propriety of the present method for evaluating the pain produced by i.m. injection of antibiotics, since the present results with rabbits are in agreement with those with humans.

ACKNOWLEDGMENTS. The authors would like to thank Ms. Sanae Moriyama for her technical assistance.

REFERENCES

Cahn, M.M. and Levy, E.J. (1972). A study of the local reaction to intramuscular disodium carbeni-
Shiro TACHIKAWA and Akio TACHIBANA


narcotic analgesics which block visceral pain evoked by intra-arterial injection of bradykinin


trypan blue and skin-twitch tests for measuring local tissue toxicity. Toxicol. Appl. Pharmacol.,
1, 73-86.

Manten, A. (1972) : Antibiotic drugs. In “Side Effects of Drugs vol. VII” (Meyler, L. and Herxhe-

Maurice, P. N., Riess, W., Welke, A. and Amson, K. (1973) : Celospor (C 36278-Ba), ein neues
Antibiotikum aus der Cephalosporinreihe : Pharmakokinetik und klinische Prüfung. Schweiz. med.
Wschr., 103, 718-724.

trical measurement of additiveness of additives for making painless injectable solutions. The


broad spectrum penicillin, PC-455. II. Antimicrobial and biological studies. “15th Interscience
Taira, N., Nakayama, K. and Hashimoto, K. (1963) : Vocalization response of puppies to intra-arterial
administration of bradykinin and other algesic agents, and mode of actions of blocking agents.