Pharmacodynamics and Potential Toxicity of Intranasally Administered Dipyrone

Shirui MAO,* Shiling YANG, and Dianzhou BI

College of Pharmacy, Shenyang Pharmaceutical University; Wenhua Road 103, Shenyang, 110016, China.
Received October 19, 2005; accepted March 17, 2006

Dipyrone is a non-narcotic analgesic and antipyretic drug used in both pediatric and adult patients. Dipyrone solution can be used intranasally as an antipyretic agent for infants. However, dipyrone is not stable in liquid state. Therefore, a stable dipyrone formulation was developed and the antipyretic effect of the formulation was studied after intranasal administration in rabbits and rats, respectively. To guarantee dose accuracy in animal studies, effect of dose volume on the distribution of dipyrone solution in rabbit nasal cavities were studied, using gentian violet as an indicator. Animal fever model and intranasal administration methods were established. In addition, the potential toxicity of the dipyrone formulation was studied. It was shown that the nasal volume of rabbits is large enough to hold 100 μl solution. After intranasal administration, improved pharmacodynamics was obtained with the new developed dipyrone formulation compared to the normal dipyrone solution, and significantly decreased body temperature was observed 10 min after dosing. The toxicity was negligible. In conclusion, the dipyrone formulation is effective and safe for clinical medication.

Key words dipyrone; nasal mucosa; pharmacodynamics; toxicity

For systemic medication, drugs are traditionally administered by oral and parenteral routes. However, in many instances, oral administration is unsuitable if a drug is significantly degraded in the gastrointestinal tract or is considerably metabolized by the first-pass effect in the liver. In addition, the parenteral route can be undesirable or impractical if a drug is intended for treatment of children diseases. An alternative route of administration would be certainly preferable. Compared to other non-parenteral routes, such as buccal, rectal, transdermal and vaginal, intranasal administration has many advantages, such as rapid absorption, comparatively high bioavailability, patient compliance, bypass of first-pass hepatic metabolism, bypass of the blood brain barrier (BBB) and therefore targeting of the central nervous system (CNS), reducing systemic exposure and thus systemic side effects. Both macromolecules and small molecules are extensively studied for nasal delivery.

Dipyrone is a non-narcotic analgesic and antipyretic drug, which is used in both pediatric and adult patients. It was introduced into clinical practice in 1922 and is still in use in many countries. Agranulocytosis, dipyrone’s most serious and potentially fatal adverse effect, has led to its withdrawal in several countries. However, this issue has been criticized by many other authors. It was reported that agranulocytosis is subject to geographical variability, ratio with at risks ranging from 0.8—23.7.5 In many countries dipyrone is still widely used in children and adults and even as an over-the-counter (OTC) preparation.5 Due to its strong analgesic effect, available parenteral formulation and low cost, dipyrone is widely used in Europe and South America. Recently, additional beneficial effects of dipyrone (vascular smooth muscle relaxant, antiapoptotic, and antiinflammatory) have been reported and increased the interest in this compound.5

Currently dipyrone is included in the pharmacopoeias of four countries, namely China, European, Japan and Poland. It is noted in the Chinese pharmacopoeia that 20% dipyrone solution can be used intranasally as an antipyretic agent for infants. Its effectiveness and convenience have been demonstrated in clinical.5 However, dipyrone is not stable in liquid state indicated by yellow color, which limited its extensive application.

In order to solve this problem, a stable dipyrone formulation was developed based on the stability investigation and formulation optimization. Briefly, using a colorimetry method, influence of pH, sorts of stabilizers on the stability of 20% dipyrone solution was studied. It was shown that 20% dipyrone solution was more stable in the pH range of 5.0—6.3 and addition of ethylene diamine tetraacetic acid (EDTA) and sodium hydrogen carbonate will increase the stability further. The color of pure dipyrone solution changed to yellow 4—6 h after preparation due to oxidization. In contrast, for the optimized dipyrone formulation only slight yellow appeared after 7 d storage at room temperature, which is sufficient for clinical use since the new developed dipyrone formulation contains two components, soluble tablet and solvent, and the solution can be prepared before use. The pharmacodynamics and potential toxicity of the newly developed dipyrone formulation were investigated in two animal models after intranasal administration, providing further evidence for clinical application. No similar research was found in available literatures to the best of our knowledge. In addition, effect of dose volume on the distribution of drug solution in the nostril was studied to guarantee the dosing accuracy in the animal experiment.

MATERIALS AND METHODS

Materials Dipyrone was purchased from Shandong Xinhua Pharmaceutical Factory, China. Typhoid, paratyphoid triple vaccines were purchased from Institute of Biological Product, The Ministry of Health, Wuhan, China. The suspension of beer yeast was a kind gift from Shenyang Beer Factory. All other chemicals used were of analytical reagent grade or higher from commercial suppliers.

Animals Adult male New Zealand-derived white rabbits weighing 2.0—3.0 kg, male Wistar rats weighing 300±25 g, were purchased from the Animal Center of Shenyang Pharmaceutical University. All animals were housed at a constant temperature of 22±2°C and given food and water ad libitum.

To whom correspondence should be addressed. e-mail: mao@staff.uni-marburg.de © 2006 Pharmaceutical Society of Japan
temperature of 20±5 °C, animal food and water were allowed ad libitum. All experimental protocols described in this study were approved by the Ethics Review Committee for Animal Experimentation of Shenyang Pharmaceutical University.

**Distribution of Dipyrone Solution** Four rabbits were restrained in the rabbit boxes and the heads kept in a supine position. Different volumes of dipyrone (20% with 0.2% gentian violet), 20, 50, 70 and 100 μl, were administered into the right and left nostrils via a polyethylene tube inserted into 10 mm. The rabbits were sacrificed by decapitation 5 min after dosing, and the mandible, brain and excess soft tissue removed. The nasal cavity was opened along the midline septum and drug solution distribution was observed and photos taken. Additionally, three other rabbits were used for elimination study. Briefly, the first rabbit was sacrificed immediately after dosing 50 μl of drug solution in each nostril, while the other two were sacrificed 2 h after administration. The nasal cavity was opened as aforementioned and compared with that of the first rabbit.

**Preparation of Nasal Formulations** All drug solutions were prepared in pyrogen-free glassware that was heated at 115 °C for 5 h before use. All solutions were passed through 0.22 μm Millipore bacterial filters prior to administration. The composition of the tested dipyrone formulation is as follows: 20% dipyrone, 3% polyvinyl pyrrolidone as viscosity-enhancing agent (as adhesive in the soluble tablet), 0.2% sodium hydrogen carbonate as antioxidant and 0.05% (EDTA) as chelator. The pH was adjusted to 5.5. The control is 20% dipyrone solution without any excipients. All solutions were prepared freshly.

**Establishment of Animal Models** The antipyretic activity of dipyrone after intranasal administration was tested in two different animal models, rabbits and rats respectively.

New Zealand-derived white rabbits were fasted overnight and weighed before experiment. Only rabbits with body temperature in the range of 38.7—40.3 °C and the difference in two measurements less than 0.2 °C were used. The rectal temperature of each animal was allowed to stabilize for at least 90 min before experimentation. The fever model was established by injecting typhoid, paratyphoid triple vaccines of 36.6—38.3 °C and the difference in two measurements less than 0.2 °C were used. Rats with body temperature increase >0.8 °C were divided into four groups randomly, that is, excipients group, dipyrone small dose group (90 mg/kg), dipyrone large dose group (180 mg/kg) and dipyrone control group (180 mg/kg) (n=10). The dose was selected based on preliminary experiments. The administration volume is 20 μl each nostril. Rats were accustomed to the dosing procedure to prevent withdrawal and defense reactions that may lead to inaccurate dosing. Other procedures are the same as described for rabbits.

**Toxicity Investigation** Acute Toxicity after Intranasal Administration: Clinically, dipyrone dose for infants is 5—10 mg/kg for injection and 10—20 mg/kg for tablet. In order to investigate the potential toxicity of intranasally administered dipyrone, 1000 mg/kg, a dose 50 times higher than the upper limit (20 mg/kg) of dipyrone tablet, was tested. Eight adult male New Zealand-derived white rabbits weighing between 20.0 and 2.5 kg were randomly divided into two groups, a test group and a control group. For the test group, dipyrone was administered intranasally at the dose of 1000 mg/kg, 0.9% NaCl was administered in the same manner as a control. After administration, the rabbits were observed for 7 d. Any changes of the animals, including weight, respiration, circulation, central nervous system and the activity of extremities were noted.

**Nasal Mucosa Irritation**: The potential irritation of the new dipyrone formulation to the nasal mucosa was investigated after a single and multiple administrations. Nine adult male New Zealand-derived white rabbits weighing between 2.0 and 2.5 kg were randomly divided into three groups, a single administration group, a multiple dosing group and a control group. In the single administration group, the dipyrone solution was administered intranasally at a dose of 150 mg/kg and the rabbits were decapsulated 24 h after administration. Nasal parts were removed and fixed with 10% formalin. In the multiple dosing groups, the rabbits were administered intranasally at a dose of 150 mg/kg for 7 d, each daily. The rabbits were decapsulated 24 h after the last administration. Histological changes of nasal mucosa were examined as described in the literature and compared with the control group.

**Statistical Data Analysis** Statistical comparisons were performed using the Student’s t test for two groups and a one-way ANOVA for multiple groups. A value of *p*<0.05 was considered to be indicative of statistical significance.

**RESULTS AND DISCUSSION**

**Effect of Dose Volume on the Distribution of Dipyrone Solution** Due to the structural characteristics of the nasal
cavity and their role of transferring drugs from the nasal membrane into systemic circulation, drug distribution in the nasal cavity affects the efficiency of nasal absorption. Application of a large volume of solution gives a good distribution over the nasal cavity, whereas a few drops give only unsatisfactory results. However, solution leakage should be taken into consideration when large volume was used. In order to find the best volume for animal experiment, drug distribution in the nasal cavity and in particular the possibility of leakage in the posterior region at relative large volume was investigated using different volume of dipyrone solution. For the ease of observation, 0.2% gentian violet was added into the solution as an indicator.

Effect of dose volume on the distribution of dipyrone using gentian violet as an indicator is shown in Fig. 1. The deposition area increased gradually with increasing volume. It shows that the nasal surface is large enough to hold 100 µl solution in each nostril with little leakage. When the volume was less than 70 µl, all the solution was retained in the nasal cavity. Fifty microliters, which is in accordance with the literature report, was used in the pharmacological study and the dosing accuracy was guaranteed.

On the other hand, time dependent nasal distribution and elimination was investigated 5 min and 2 h after administering 50 µl of test dipyrone solution containing 0.2% gentian violet. The results are shown in Fig. 2. The purple color became weaker 2 h later, implying that the solution was probably absorbed across the nasal mucosa, or eliminated by the nasal cavity due to the mucociliary clearance.

Pharmacodynamics of Dipyrone after Intranasal Administration To study the absorption of dipyrone via the nasal mucosa, antipyretic studies were performed in non-surgically modified conscious animals. No anesthetic agents were used in this study, as they were reported to increase the nasal absorption of drugs greatly. This is probably due to the impairment of the mucociliary clearance in the deeply anaesthetized rats, together with the lack of any possible loss due to drainage and mechanical removal.

Antipyretic Effect to the Triple Vaccine Induced Rabbits: In this study, 50 µl of dipyrone solution was delivered into each nostril and a total volume of 100 µl per rabbit was administered. This administration protocol was selected because the site of drug deposition within the nose is not only dependent upon the dosage form but also the dose volume. Previous studies showed that the bioavailability of intranasal desmopressin from a 2×50 µl dose was 20%, which represented a marked increase over 11% found with 1×50 µl spray or 9% obtained from the 1×100 µl intranasal dose. Such findings indicate that an optimal bioavailability may be obtained by administering an equal dose into each nostril.

Pharmacological responses of tested dipyrone formulations were determined after intranasal administration and compared with that of the control, as shown in Fig. 3. No sig-
significant difference between excipients and 0.9% NaCl group was found, implying that the excipients have no pharmaco-
logical activity. Body temperature decreased significantly in all the treatment groups \( (p<0.05) \) and a dose–response relationship was found. For the large dose dipyrone (150 mg/kg) group, body temperature began to decrease significantly 10 min after dosing \( (p<0.01) \) and the action lasted for 6 h. In contrast, considerably decreased temperature was found at 20 min for small dose dipyrone (75 mg/kg) \( (p<0.05) \) group and it lasted for 5 h. Regarding to the dipyrone control group, significant antipyretic effect was found at 1 h \( (p<0.05) \) and the action lasted for 4 h. No significant difference between dipyrone control and small dose groups was found \( (p>0.05) \), but they were statistically different with the large dose group \( (p<0.05) \). This indicates that at the same dose, dipyrone in the new formulation has stronger pharmacological activity.

Antipyretic Effect to Beer Yeast Induced Rats: Similarly, intranasal administration of dipyrone (90 and 180 mg/kg) induced significant body temperature decrease in rats \( (p<0.05) \) and the activity was dose dependent, as shown in Fig. 4. Compared to the 0.9% NaCl group, a remarkable body temperature decrease was observed at 10 min in dipyrone small, large dose groups \( (p<0.01) \) and control group \( (p<0.01) \). The activity lasted for more than 6 h for the large dose group, compared to 5 h for the small dose and control groups. A statistical difference was found between the small and large dose groups \( (p<0.05) \), the large dose and the control groups \( (p<0.05) \). However, no significant difference was found between dipyrone small dose and control groups \( (p>0.05) \), implying an enhanced activity of dipyrone in the new formulation, an identical conclusion drawn from the rabbit experiment.

Apparently, the above animal experiments demonstrated that the pharmacodynamics of the tested dipyrone solution was considerably stronger than that of the control at the same dose. This could probably be explained by the viscosity-enhancing agent in the tested solution, which prolonged the retention time of dipyrone on the nasal mucosa to some extent, leading to enhanced absorption. Regarding the influence of viscosity-enhancing agents on the intranasal absorption of drugs, reports from literatures were quite controversial. Ikeda et al.\(^{13}\) showed that the bioavailability of penicillamine increased from 11.7 to 20% after adding 2% hydroxypropyl cellulose (HPC) in the solution. Hussain\(^{14}\) showed that the blood levels of propranolol increased remarkably after adding 3% (w/v) methyl cellulose (MC) in the nasal solution. However, Harris et al.\(^{15}\) indicated that viscosity-enhancing agent could only decrease the drug diffusion rate, which results in a longer retention time and slower clearance along the nasopharynx, but no significant improvement in bioavailability was demonstrated. Anyhow, our experiment did demonstrate that intranasal absorption of dipyrone could be enhanced by increasing the viscosity of the administered solution. It was clear that the contribution of improved stability couldn’t be excluded. However, since the solutions were prepared freshly before the experiment, and drug content decrease was less than 2%, therefore, it was not a main contributing factor. We assume that the influence of viscosity-enhancing agent on the intranasal absorption of dipyrone was related to the properties of the drug substance, such as molecular weight, lipophilicity. Dipyrone is a strong hydrophilic drug with a low molecular weight (351.3). Its absorption mechanism via the nasal mucosa is passive diffusion.\(^{16}\) Increasing viscosity prolonged the contact time with the nasal mucosa, leading to enhanced absorption.

On the other hand, it was noted that a significant rectal temperature decrease was observed at 10 min after administering large dose dipyrone in both animal models. In contrast, for the dipyrone control group, a remarkable activity was found at 10 min in rats \( (p<0.01) \) and 1 h in rabbits \( (p<0.05) \). This could probably associate with interanimal variability.

**Toxicity of the Dipyrone Nasal Formulation** The effectiveness of dipyrone was demonstrated in animal experiments. However, the safety of the formulation is of special importance before clinical application. Therefore, the acute toxicity of the dipyrone formulation was investigated with an extremely high dose. Fortunately, no abnormality was found during the 7 days’ observation. The hair color of the rabbits was glossy, the respiration was normal, and no apparent excitement or restrain behavior was observed. All the activities were normal without death. No significant weight difference was detected \( (p>0.05) \).

In addition, morphology of the nasal mucosa after irritation investigation is shown in Fig. 5. In sections prepared from undosed animals, a continuous epithelial layer covered all surfaces of the rat nasal cavity (Fig. 5A). After a single and multiply dosing, the nasal cavities were covered by an intact, undamaged epithelium layer (Figs. 1B, 1C), comparable to that of the undosed control (Fig. 5A), indicating that the formulation has no or slight irritation to the nasal mucosa.

In summary, these results showed that the new developed dipyrone formulation was almost nontoxic and was suitable for clinical use.

**Absorption of Dipyrone across the Nasal Mucosa** Pharmacodynamics studies showed that dipyrone had strong antipyretic effect after intranasal administration both in rabbits and rats, implying that dipyrone was absorbed into the systemic circulation via the nasal mucosa. This point was in agreement with our previous investigation performed with in situ nasal recirculation method.\(^{16}\) This technique involves
perfusion of the drug solution through the nasal cavity of the rat at a constant rate for a certain period of time. It proved that a large amount of dipyrone was absorbed via the nasal mucosa and the absorption mechanism was passive diffusion. The average absorption rate constant was $0.02219 \text{ min}^{-1}$. It has been reported that the in situ nasal absorption studies could be used to predict approximately in vivo nasal absorption of drugs. This point was demonstrated here in this paper.

REFERENCES