Drug Development and Industrial Pharmacy, 2010; 36(4): 490–496

informa
healthcare

ORIGINAL ARTICLE

Preparation of nasal temperature-sensitive in situ gel of *Radix Bupleuri* and evaluation of the febrile response mechanism

En Chen, Jun Chen, Shi-lei Cao, Qi-zhi Zhang and Xin-guo Jiang

Department of Pharmaceutics, School of Pharmacy, Fudan University, Shanghai, PR China

Abstract

Objective: This study developed a nasal temperature-sensitive in situ gel system for *Radix Bupleuri*. *Method*: Using 20% Poloxamer 407 as the gel base and 6% PEG 4000 adjusting the gelation temperature. *Results*: The system is liquid at 4°C. It can change its phase to gel above 30°C, which is close to the temperature in nasal cavity. The antipyretic effect produced by *Radix Bupleuri* in situ gel formulation was investigated in fevered rabbits. The results show that it can prolong the effective time to 24 hours compared with 4–6 hours in *Radix Bupleuri* intranasal solution. The antipyretic response mechanism was researched by evaluating the relationship between body temperature and concentrations of cyclic adenosine monophosphate in cerebrospinal fluid. The results showed that the two parameters were positively correlated ($r = 0.9435$, $P < 0.05$). Six hours later after given in situ gel, the concentrations of cAMP were significantly lower than those in the solution group. It confirmed that temperature-sensitive *Radix Bupleuri* in situ gel applied in the nasal sprays had a longer residence and release time. *Conclusion*: *Radix Bupleuri* nasal temperature-sensitive in situ gel has a higher medical effect and a longer effective time. Compared to the traditional nasal spray, it is more applicable for the treatment of fever.

Key words: *cAMP; fever cooling; in situ gel; nasal delivery system; pharmacodynamics; poloxamer;* Radix Bupleuri

Introduction

Radix Bupleuri is defined as a root or the entire grass of umbelliferae bupleurum plant. It has been clinically used to treat upper respiratory tract infection, pneumonia, flu, and other fever symptoms[1]. The essential oil extracted from the herb is regarded as an effective product, which has been formulated into a variety of dosage forms such as *Radix Bupleuri* injection, major *Radix Bupleuri* decoction, and minor *Radix Bupleuri* decoction[2]. But these preparations have some deficiencies. For example, *Radix Bupleuri* injection makes patients suffer from ache and *Radix Bupleuri* decoction has first-pass effect. Some published results showed that *Radix Bupleuri* injection given by intranasal administration had significantly reduced fevers in 97% of 100[3]. Advantages associated with the nasal route over oral administration include a higher bioavailability due to no first-pass hepatic metabolism, a great acceptability to injection, and an allowance to self-medication. Our group has already proposed a *Radix Bupleuri* nasal spray as an aqueous solution[2]. However, because of mucociliary clearance, drug in a form of solution condition usually has a very short residence time in the nasal cavity with a clearance half-life of 15 minutes[4,5]. Consequently, only a short-term efficacy remains, and thus a frequent dosing regimen was needed.

In situ gel is referred to a kind of preparation in a form of solution condition; and with the changes of physiological environment of administration position, the phase changes and forms a gelatinous semi-solid preparation[6]. Because it is benefited from merits of both solution and gel, it has a broad application prospect in research of drug carrier. The favorite types of in situ gels

Address for correspondence: Dr. Xin-guo Jiang, Department of Pharmaceutics, School of Pharmacy, Fudan University, Room 606, Ke Yan Building, No. 826 Zhang Heng Road, Shanghai 201203, PR China. E-mail: xgjiang@shmu.edu.cn.

(Received 25 Mar 2009; accepted 16 Aug 2009)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.3109/03639040903264371

are temperature-sensitive[7], ion-sensitive[8], and pH-sensitive[9]. With the study in depth, some new types such as light-sensitive[10,11], electricity-sensitive[12], magnetic-sensitive, and others have become popular. This article reports a method to prepare *Radix Bupleuri* essential oil into the temperature-sensitive in situ gel. Also it represents a comprehensive research on prescription, preparation, nasal cavity irritation, pharmacodynamics, and so on.

Poloxamer 407 as an effective temperature-sensitive polymer is composed of hydrophilicity polyoxyethylene (PEO) and hydrophobicity polyoxypropylene (PPO). Aqueous solution of poloxamer was gelled on warming to body temperature. It has been reported to be used in the preparation of in situ gel rectal administration. So it is used as a type of in situ gel material in this study.

The mechanism of febrile response is also investigated in this study. Some researchers reported that microinjections of cyclic adenosine monophosphate (cAMP) agonists into the preoptic region of the anterior hypothalamus (POA) increase body temperature[13,14]. It means that an increase in cAMP level in the POA is associated with fever. Therefore, we suggested that measuring the content of cAMP in cerebrospinal fluid (CSF) can reflect the state of the body heat and indirectly evaluate the effect of the *Radix Bupleuri* in situ gel.

Materials and methods

Materials

Radix Bupleuri was purchased from Anguo Changan Pharmaceutical Co. Ltd. (Hebei, China). Poloxamer 407 (P407, Lutrol F127) was kindly donated by BASF Co. (Ludwigshafen, Germany) Pharmasolve was obtained from International Specialty Products Co. (Calvert, USA). ^{125}I-cAMP RIA Kit was supplied by Shanghai Chinese Medical University (Shanghai, China). All other reagents were of the highest grade commercially available.

Extraction of essential oil

The essential oil was extracted from *Radix Bupleuri* by a modification of steam[2]. In brief, 450 g *Radix Bupleuri* was cut into several small pieces and dipped into 2700 mL saturated saline water for 12 hours. And then the steam distillation step was performed with 1350 mL distilled solution collected and saturated with NaCl. Subsequently, this solution was directly distilled again to collect 200 mL second-distilled solution, to which NaCl was added until it was saturated. At the end of the distillation step, 100, 100, 50 mL ethyl ether was applied in a sequent order to extract the essential oil from the second-distilled solution, and the collections were mixed with their remaining water eliminated with anhydrous Na_2SO_4. Finally, ethyl ether was removed under an air flow, thus gaining about 0.2 mL essential oil. The process of nitrogen drying should be maintained at low temperature and blowing as much quickly as possible to shorten the time. The essential oil should be preserved in confined bottles.

Preparation of Radix Bupleuri *in situ gel*

In situ gel was prepared on a weight/weight basis by means of a cold method described by Schmolka and Biomed[15]. Appropriate amount of P407 was prepared in a refrigerator as a gel base. We kept the temperature at 4°C and added a 15 mL amount of this P407 solution into a container. A thermometer with the variance smaller than 0.1°C was inserted. The mercury ball was completely immersed into the gel solution. Then the container was put into a water bath and slowly heated. A rotating viscometer (NDJ-5S; Shanghai Geology Apparatus Factory, Shanghai, China) recorded the gel solution's viscosity in various temperatures. We named the temperature at which the viscosity changes dramatically as the gelation temperature. The gelation temperature of the polymer is related to the polymer concentration and the ratio of PEO–PPO block. When the concentration is still, the PEO–PPO block ratio can significantly affect the gelation temperature. On this study, we use PEG 4000 to adjust the PEO–PPO block ratio to control the gelation temperature.

Based on the preliminary study, a mixture of 10% (v/v) Pharmasolve and 2% (v/v) Tween 80 was mingled to dissolve the essential oil extracted from 450 g *Radix Bupleuri* and stirred for 5 minutes. Afterward, benzylalcohol 0.15% (v/v) and vitamin C 0.1% (v/v) were added as a preservative and an antioxidant, which was stirred again for 2 minutes. A certain amount of P407 and PEG 4000 was added and increased to 30 mL with distilled water. The solution was placed in a refrigerator until a clear, homogenous liquid was formed. In these formulations, poloxamer preserved its ability to form a gel upon warming up.

In vitro release study

In vitro release of *Radix Bupleuri* in situ gel was investigated by using dialyzing method (dialysis membrane 12,000 MWCO) against artificial nasal fluid, which is composed of NaCl (0.79%), $NaHCO_3$ (0.26%), KCl (0.37%), and $CaCl_2$ (0.05%)[16]. The formula (3 mL) that was sealed in a dialysis membrane was transferred to a glass cylinder with 120 mL artificial nasal fluid. Then the glass cylinder was attached to a dissolution apparatus (ZRS-89; Tianjin Radio Factory, Tianjin, China) at the

temperature of 37°C. The system was allowed to rotate at a constant speed (100 rpm). At 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours, aliquots were withdrawn and replaced by an equal volume of the receptor medium. The drug concentrations in the withdrawn samples were determined spectrophotometrically[17] at λ 278 nm using UV-visible double beam spectrophotometer (UV-2401PC; Shimadzu, Kyoto, Japan). The results were calculated as the means of three runs.

Nasal ciliotoxicity

Nasal ciliotoxicity studies were carried out by using in situ toad palate model[18]. In this experiment, the upper palate of toad was exposed to and treated with 0.5 mL test *Radix Bupleuri* in situ gel for 4 hours and then rinsed quickly with normal saline (NS). Finally, the palate was dissected and the mucocilia was immediately examined with an optical microscope. NS and sodium deoxycholate (one of serious nasal ciliotoxicity agents, 1% (w/v) solution) were used as negative and positive control.

Antipyretic effect on rabbits

Animals

The New Zealand-derived white male rabbits (2.0 ± 0.2 kg, Shanghai Experimental Animal Center, Chinese Academy of Sciences, Shanghai, China) were housed in a controlled environment and were given access to food and water freely. The animal experiment was carried out in compliance with the protocol of Animal Use and Care by Medical Center of Fudan University.

Animal handling and dosage regimen

Two days before the experiment, the animals were trained according to a handling procedure so that at the day of experiment the animals did not present any handling-induced rise in body temperature. Pyrexia was induced in rabbits with a subcutaneous injection of 0.15 mL/kg turpentine and their temperatures were recorded 16 hours after the injection to determine the rabbits' pyretic responses. The rabbits whose temperatures had risen over 1°C were selected and divided into three groups randomly for the antipyretic test (n = 8). After that, their temperatures were recorded 1 hour prior to drug administration and served as predrug control[19]. The test formula was bottled in 3 mL nasal spray bottle (Pfeiffer Co., Assalar, Germany) with a quantitative spray pump (0.1 mL/spray). Before administration, the pump should be pressed several times until the droplets from the pump are well distributed. Then put the nozzle into nasal cavity, pressing rapidly at both sides of nasal cavity of the rabbit, keeping the nose upturned for seconds to prevent in situ gel flowing out before it forms to gel.

Table 1. Schedule for fever test in rabbits.

Group	Name	Method	Dosage (mL)
1	NS	i.n.	0.2
2	*Radix Bupleuri* solution	i.n.	0.2
3	*Radix Bupleuri* in situ gel	i.n.	0.2

i.n., intranasal administration.

Schedule for the fever test was showed in Table 1. The intranasal solution[2] that was obtained by our group before is used as comparison.

The temperatures of rabbits were recorded at predetermined time points after the administration.

Antipyretic mechanism test

Endotoxin-induced fever model

In animal screening method as above, each rabbit was injected intravenously *Escherichia coli* endotoxin (ET) solution 10 EU/kg[20,21]. Five hours later, they were injected again with the same dose of ET solution. Their body temperature was recorded at predetermined time points for 10 hours.

CSF serial sampling and dosage regimen

Rabbits were anesthetized with an intravenous injection dose of 30 mg/kg sodium pentobarbital and fixed onto a stereotaxic apparatus (CSHAUBLIN 5 II 52–31). The skin overlying the occipital bone was incised and then the underlying muscle and tissue were bluntly dissected to expose the atlanto–occipital (a–o) membrane. A 25-gauge needle connected with a PE-10 tube was punctured into the bottom part of a–o membrane. Once the needle was punctured into cisterna magna, CSF would flow into PE tube because of the inner pressure, and then mucilage was used to fasten the needle. Before sample collection, a clamp was used to close the tube. Intravenous ET solution (10 EU/kg) was injected into ear vein after the animal was awaked. At the same time, the nasal formula was given according to Table 1. CSF (100 μL) was collected at each time point. The samples were collected in a preaccession 5-mL plastic centrifuge tube[22,23].

CSF sample collect and analysis

The CSF samples were centrifuged at 800 × g for 10 minutes, the clear solution was collected and was dried at 60°C water bath.

CSF samples were determined by ^{125}I RIA KIT, and the concentration of cAMP was measured according to KIT specification.

Statistical analysis

The results were expressed as mean ± SD. A two-sided paired or unpaired Student's t-test was applied. Differences were considered statistically significant at $P < 0.05$.

Results and discussion

Preparation of in situ gel

P407 solution would be transferred to gel when heated. And its gelation temperature increased with the P407 concentration decreasing (Figure 1). The viscosity of 15% of the P407 solution was almost steady with the change of temperature. The viscosity of 17% of the P407 solution increased, but the changes were not significant. The viscosity of P407 solution rose dramatically, more than 20%, at a certain temperature point (gelation temperature). After that, the change of viscosity stabilized.

When below 20%, the gel strength is low. However, above 20%, the gelation temperature is low (Figure 2). So 20% of P407 is the most suitable concentration for in situ gel administration.

PEG 4000 was added to the formula to make sure the gelation temperature was around 30°C (close to the nasal physiological environment). According to our research, 6% of PEG 4000 can rise the gelation temperature to 30°C (Table 2).

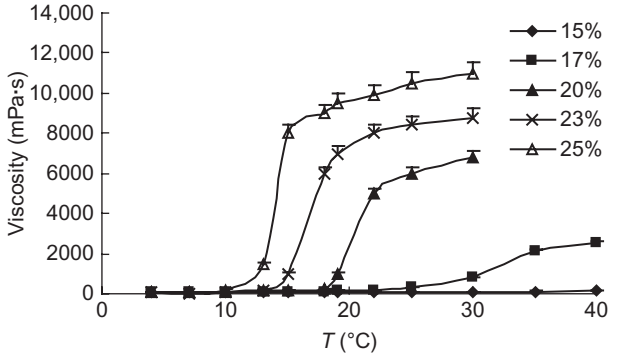

Figure 1. Viscosity of five kinds of P407 solutions.

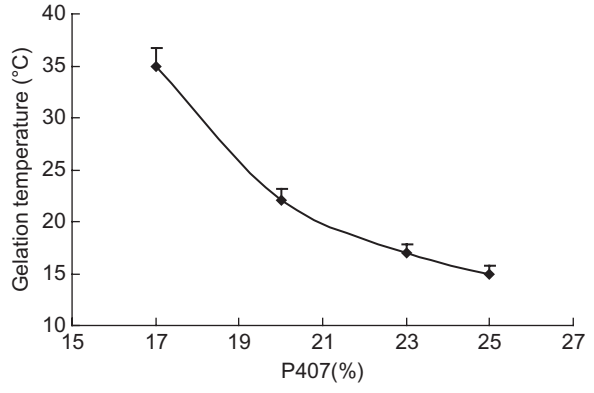

Figure 2. The gelation temperature of different concentrations of P407.

Table 2. 20% P407 gelation temperature of in situ gel, with different content PEG 4000.

PEG 4000	2%	3%	4%	5%	6%	7%
Gelation temperature	21°C	22°C	23°C	26°C	30°	–

'–' means cannot change to gel phase at any temperature.

The ability of P407 matrix-based against the environmental dilution (antidilution effect) is weak. For example, if 20% of the P407 solution is fully mixed with artificial nasal fluid (1:1 v/v), the viscosity of in situ gel at physiological temperature decreased significantly. However, when we sprayed the *Radix Bupleuri* in situ gel into rabbits' nasal cavity and incised organization 0.5 hour after administration, we observed that the gel definitely formed in nasal cavity. The reasons may be (i) because of the high local concentration of in situ gel, in situ gel solution that has greater viscosity than normal nasal fluid cannot be diluted easily, and (ii) the phase switching procedure of in situ gel is fast. The gel has formed before it is fully diluted by nasal fluid.

A lot of polymers are reported as additives adjusting the ratio of PEO–PPO block, such as Poloxamer188[24], PEG[25]. There are also reports showing that comparing with PEG, addition of Poloxamer188 can enhance the antidilution effect used in the eye drug delivery system. However, taking the larger amount of P188 into account, polymers in solution condition have a high degree of viscosity. It cannot be sprayed from pumps easily. Concerning the different internal environments between eyes and nasal cavity, we used PEG 4000 as additives. The results show that it can form the gel in nasal cavity.

Despite the flowability of in situ gel at low temperature is positive, the viscosity is still too high compared with the common solution. Therefore, it is important to choose an appropriate gel-pump for usage of nasal spray. But the droplets are still larger than common liquid spray. How to continue to reduce the viscosity of preparation in nonphysiological conditions is under further investigation.

In vitro release study

The in vitro release study results are presented in Figure 3. It revealed that about 95% of the drugs were released within 8 hours. An initial rapid release of the drug occurs, followed by a subsequent slow and sustained release. The water layer is responsible for these phenomena. Water molecules infiltrate into gel to form a boundary layer, which loosely pile up on the gel surface. Therefore, the boundary layer makes the outside drug release directly. However, it delays the inside gel from dissolving and drug releasing.

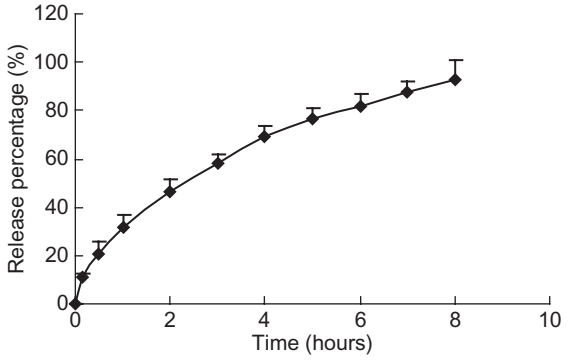

Figure 3. Release of *Radix Bupleuri* nasal temperature-sensitive in situ gel.

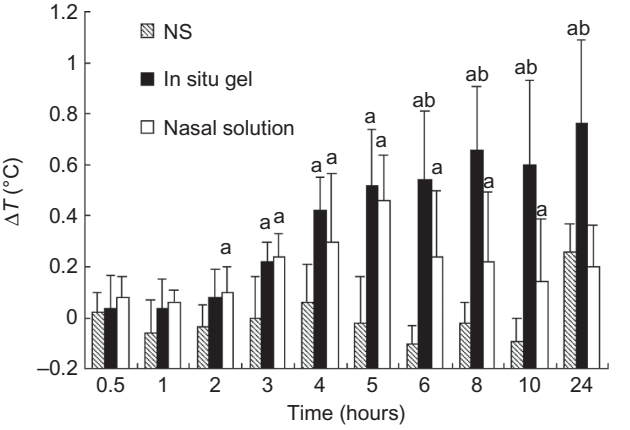

Figure 5. Effect of *Radix Bupleuri* temperature-sensitive in situ gel and solution. [a] significantly different from NS, $P < 0.05$; [b]significantly different from solution, $P < 0.05$.

Nasal ciliotoxicity

Optical microscopic results showed that there were a great number of cilia at a fast beating rate on the edge of the mucosa treated with the NS (negative control group) (Figure 4a), and the beating lasted for about 10 hours after the palate was dissected. The in situ gel group had the same phenomena under the microscope. The cilia beating lasted for about 9 hours, almost the same time as NS group (Figure 4b). Compared with the sodium deoxycholate group (positive control group) (Figure 4c), there were no cilia on the edge of the mucosa. Based on the results above, we could conclude that both the essential oil and the excipients possess no serious nasal ciliotoxicity.

Antipyretic effect

In this part of study, giving the same dose of *Radix Bupleuri* nasal solution and in situ gel, the effect of two tests was different (Figure 5). The solution group reached the best cooling effect 5 hours after administration. The temperature declined in 0.5°C. However, its cooling duration was only about 6 hours. The solution group and the in situ gel group had almost the same effect in the first 5 hours after administration. But compared to the solution group, the effective time duration of in situ gel could be extended to 24 hours and had the greatest temperature declining in 0.8°C.

It confirmed that in situ gel was able to produce a prolonged therapeutic effect for *Radix Bupleuri*. The membrane-free dissolution test[26] showed that the percentage of the accumulation rate of gel corrosion and drug release are equivalent. The two parameters both act to follow zero-order kinetic equation. We can conclude that during gel formation, most of the essential oil was loaded into the gel phase and delivered in a sustained form. These results indicated that *Radix Bupleuri* in situ gel had a significant and longer antipyretic capacity in rabbits at doses used above.

Antipyretic mechanism test

To determine the relationship between fever temperature and concentrations of cAMP, these two parameters were obtained from the studies. The concentration of cAMP (Figure 6) in CSF reached its maximum at 1 and 6

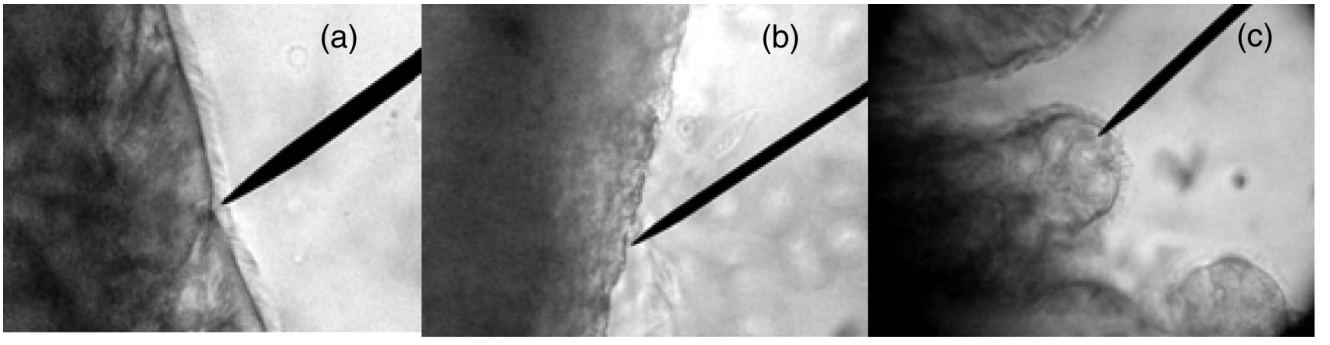

Figure 4. Picture of toad upper mucosa pilus. (a) NS as negative control group; (b) sodium deoxycholate as positive control group; (c) *Radix Bupleuri* in situ gel as test group.

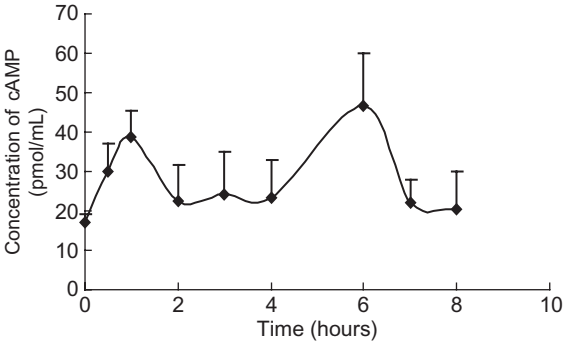

Figure 6. Concentration of cAMP in ET-rabbits' CSF.

hours (reinject ET at 5 hours). Compared to the fever curves (Figure 7), the two shapes were similar. Correlation test showed that the two parameters were positively correlated (r = 0.9435, P < 0.05). Therefore, the concentration of cAMP in CSF could reflect the temperature level of fever rabbits.

A comparison of the antipyretic effect between *Radix Bupleuri* nasal solution and in situ gel through the analysis of cAMP contained in CSF was conducted (Figure 8). One hour after intranasal administration, the content of cAMP in both solution and in situ gel groups decreased.

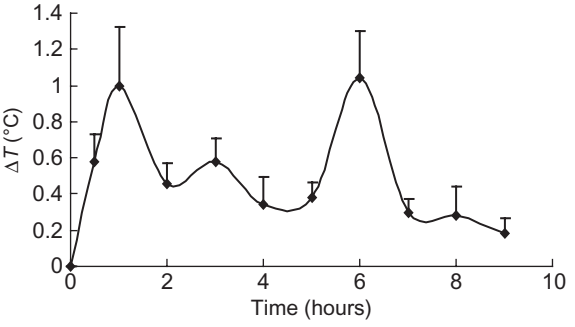

Figure 7. ET rabbit fever curve.

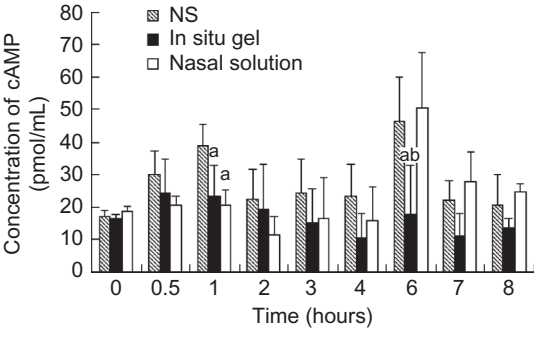

Figure 8. Concentration of cAMP in three kinds of preparation. [a]significantly different from NS, P < 0.05; [b]significantly different from solution, P < 0.05.

The decreasing level had no significant difference between these two groups (P > 0.1). Six hours after administration, the solution group resumed its high level of cAMP. However, the in situ gel group still kept the low level. Therefore, it indicated that in situ gel could prolong the duration of drug effect.

Many studies done by our group showed that intranasal administration had a set of good characteristics for brain drug delivery[27–29]. At this part of experiment, the concentrations of cAMP in CSF were significantly lower than negative control group after given *Radix Bupleuri* in situ gel through intranasal administration. It further confirmed that the nasal administration had a special access for drug delivery through the nasal cavity. It had a set of certain characteristics for brain drug delivery.

Conclusions

In this study, it was proved that a *Radix Bupleuri* nasal temperature-sensitive in situ gel, which was composed of essential oil, P407/PEG 4000 (20/6%, wt/wt), and Tween 80/Pharmasolve (2/10%, v/v), had optimum adhesion properties and no serious nasal ciliotoxicity. The animal experiment suggested that *Radix Bupleuri* in situ gel could be more effective than the nasal solution. The antipyretic mechanism test indicated that the concentration of cAMP in CSF could reflect the degree of pyrexia. Results showed that cAMP of in situ gel group was longer than the contrast group. In conclusion, the in situ gel system is a promising approach of intranasal delivery of *Radix Bupleuri* to achieve the therapeutic effect improvement.

Acknowledgment

This work was supported by National Basic Research Program of China 973 Program (no. 2007CB935800), National Natural Science Foundation of China (no. 30171112), and National Key Program of Pharmaceutical Creation and Development (no. 2009ZX09310–006).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

References

1. Chang HM, But PHH. (1987). Pharmacology and application of Chinese materia medica. Singapore: World Scientific Publishing.

2. Xie YL, Lu W, Cao SL, Jiang XG, Yin M, Tang WL. (2006). Preparation of *Bupleurum* nasal spray and evaluation on its safety and efficacy. Chem Pharm Bull, 54:48–53.
3. Cao AX. (1999). Cases of antipyretic effect by *Radix Bupleuri* nasal solutions. J Pract Tradit Chin Med, 15:15–6.
4. Gizurason S. (1993). The relevance of nasal physiology to the design of drug absorption studies. Adv Drug Deliv Rev, 11:329–47.
5. Illum L, Jorgensen H, Bisgaard H, Krogsgaard O, Rossing N. (1987). Bioadhesive microspheres as a potential nasal drug delivery system. Int J Pharm, 39:189–99.
6. Wei G, Liu WY, Zheng JM. (2004). Diffusion behaviors of drugs in thermosensitive in situ gels. Acta Pharm Sin, 9:232–5.
7. Desai SD, Blanchard J. (1998). Evaluation of Pluronic F127 sustained-release ocular delivery systems for Pilocarpine using the albino rabbit eye model. Pharm Sci, 87:1190–5.
8. Cohen S, Lobel E, Trevgoda A. (1997). A novel in situ-forming ophthalmic drug delivery system from alginates undergoing gelation in the eye. J Control Release, 44:201–08.
9. Srividya B, Cardoza RM, Amin PD. (2001). Sustained ophthalmic delivery of Ofloxacin from a pH triggered in situ gelling system. J Control Release, 73:205–11.
10. Hatefi A, Amsden B. (2002). Biodegradable injectable in situ forming drug delivery systems. J Control Release, 80:9–28.
11. Hubbell JA, Pathak CP, Sawhney AS, et al. (1995). Photopolymerizable biodegradable hydrogels as tissue contacting materials and controlled release carriers. US patent no. 5410016.
12. Okuzaki H, Osada Y. (1994). Electro-driven chemomechanical polymer gel as an intelligent soft material. J Biomater Sci Polym Ed, 5:485–96.
13. Philipp DWK. (1976). Evidence for the involvement of adenosine 3′,5′-cyclic monophosphate in fever genesis. Pfügers Arch, 362:223–7.
14. Willies GH, Wolf CJ, Rosendorff C. (1976). The effect of an inhibitor of adenylate cyclase on the development of pyrogen, prostaglandin and cyclic AMP fevers in the rabbit. Pfügers Arch, 367:177–81.
15. Schmolka IR, Biomed J. (1972). Fever and anapyrexia in systemic inflammation: Intracellular signaling by cyclic nucleotides. Mater Res, 6:571.
16. Lorin MI, Gaerlan PF, Mandel ID. (1972). Quantitative composition of nasal secretions in normal subjects. J Lab Clin Med, 80:275–81.
17. Mai M, Samar M, Nahed DM, Seham SAE. (2008). Ocular poloxamer-based ciprofloxacin hydrochloride in situ forming gels. Drug Dev Ind Pharm, 34:744–52.
18. Jiang XG, Cui JB, Fang XL, Wei Y, Xi NZ. (1995). Toxicity of drugs on nasal mucocilia and the method of its evaluation. Acta Pharm Sin, 30:848–53.
19. Cao SL, Chen E, Zhang QZ, Jiang XG. (2007). A novel nasal delivery system of a Chinese traditional medicine, *Radix Bupleuri*, based on the concept of ion-activated in situ gel. Arch Pharm Res, 30:1014–9.
20. Du Y, Huang QQ, He ZM, Li GM. (2003). Studies on rabbit's tolerance to bacterioendotoxin's pyrogen in pyrogen test. Lab Anim Sci Manage, 20:13–5.
21. Goudah A, Mouneir SM, Shim JH, Abd EAA. (2006). Influence of endotoxin induced fever on the pharmacokinetics of intramuscularly administered cefepime in rabbits. J Vet Sci, 7:151–5.
22. Van DBM, Romeijn SG, Verhoef JC, Merkus FW. (2002). Serial cerebrospinal fluid sampling in a rat model to study drug uptake from the nasal cavity. J Neurosci Methods, 116:99–107.
23. Shi ZQ, Zhang QZ, Jiang XG. (2005). Enhancement of systemic and CNS delivery of meptazinol hydrochloride by intranasal administration to rats. Acta Pharm Sin, 40:754–7.
24. Amal EK, Mona EK. (2006). Thermally reversible in situ gelling carbamazepine liquid suppository. Drug Deliv, 13:143–8.
25. Katarina E, Johan C, Roger P. (1998). Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur J Pharm Sci, 6:105–12.
26. Wei G, Zheng JM, Li SM. (2002). Studies on Thermosensitive in Situ Gels for Ophthalmic Use. China: Shenyang Pharmaceutical University.
27. Zhang QZ, Zha LS, Zhang Y, Jiang WM, Lu W, Shi ZQ, et al. (2006). The brain targeting efficiency following nasally applied MPEG–PLA nanoparticles in rats. J Drug Target, 14:281–90.
28. Chen J, Jiang XG, Jiang WM, Gao XL, Mei N. (2005). Intranasal absorption of rizatriptan—in vivo pharmacokinetics and bioavailability study in humans. Pharmazie, 60:39–41.
29. Lu W, Zhang Y, Tan YZ, Hu KL, Jiang XG, Fu SK. (2005). Cationic albumin-conjugated pegylated nanoparticles as novel drug carrier for brain delivery. J Control Release, 107:428–48.