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Preliminary communication

Dendrimers as potential drug carriers. Part II. Prolonged delivery of ketoprofen by in vitro and in vivo studies

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Abstract

Ketoprofen, a non-steroidal anti-inflammatory drug with well-known anti-inflammatory, antipyretic and analgesic properties, has low solubility in water and causes local or systemic disturbance in the gastrointestinal tract. In the present study we investigated the potential of polyamidoamine (PAMAM) dendrimers as drug carriers of ketoprofen by in vitro and in vivo studies. The in vitro release of ketoprofen from the drug–dendrimer complex is significantly slower compared to pure ketoprofen. Anti-nociceptive studies using the acetic acid-induced writhing model in mice showed a prolonged pharmacodynamic behavior for the ketoprofen–PAMAM dendrimer complex. Also, the blood level studies were investigated. We concluded that PAMAM dendrimers might be considered as a potential drug carrier of ketoprofen with a sustained release behavior under suitable conditions.

Keywords: Polyamidoamine dendrimers; PAMAM; Drug carrier; Ketoprofen

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently used drugs in the world, primarily for symptoms associated with osteoarthritis and other chronic musculoskeletal conditions [1,2]. Ketoprofen, 2-(3-benzoylphenyl)-propionic acid, a member of the NSAID, is an inhibitor of prostaglandin synthetase. It is effective in the long-term management of rheumatoid arthritis, ankylosing spondylistis, osteoarthritis and acute out, as well as mild to moderate pain and dysmenorrhea [3–5], and has been used as model drug for such investigations [6].

However, ketoprofen has been administered orally, three or four doses per day [7], its significant adverse effects limit the use of ketoprofen, which include gastrointestinal side effects [8,9] (such as dyspepsia, gastrointestinal bleeding, and even perforation), renal side effects and some additional side effects [10] (such as hypersensitivity reactions and distinct salicylate intoxication).

Previously, we have attempted to use polyamidoamine (PAMAM) dendrimers as a potential drug carrier to improve the solubility of the drug in water [11]. As poor solubility is generally related to a low bioavailability, this presents a major challenge during drug formulation [12]. In this present study, we used G5 PAMAM dendrimer to investigate the potential of PAMAM dendrimers as a carrier of hydrophobic drugs as exemplified by ketoprofen.

Dendrimers are hyperbranched, monodisperse, three-dimensional macromolecules, having defined molecular weight and host-guest entrapment properties. They allow the precise control of size, shape and placement of functional groups and combine typical characteristics of small organic molecules and polymers that result in special physical and chemical properties [13–16]. Accordingly, dendrimers have attracted increasing attention for their applications in many fields. Among them the use of dendrimers as a drug carrier in delivery systems has been of great interest [17,18].

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Polyamidoamine (PAMAM) with an ellipsoidal or spheroidal shape is one of the most-studied starburst macromolecules [13]. PAMAM has a much higher amino group density comparing with conventional macromolecules, e.g. a third generation PAMAM prepared from ammonia core has 1.24×10^{-4} amine moieties per unit volume (cubic Angstrom units) in contrast to the 1.58×10^{-6} amine moieties per unit volume of a conventional star polymer [16]. Also, PAMAM dendrimers possess empty internal cavities, where drug molecules can easily be encapsulated in the dendrimer interior. The special structure and high density of amino groups in PAMAM may be expected to have potential applications in enhancing the solubility of the low aqueous solubility drugs and as delivery systems for bioactive materials [17,18]. Drugs bound to dendrimers are at early stages of development and data on them are limited. Here, we focus on using PAMAM dendrimer as potential drug carriers, which are emerging as a promising group of safer and perhaps more effective alternatives to traditional NSAIDs.

2. Experiments

2.1. Materials

ketoprofen was purchased from Hubei Wuxue Xunda Pharmaceutical Co. (Hubei, China). Ethylenediamine, methyl acrylate, methanol (HPLC grade) were obtained from Shanghai Chemical Co. (Shanghai, China). For both in vitro and in vivo studies, distilled water was used. For high performance liquid chromatography (HPLC) studies double-distilled water was used.

2.2. Preparation of ketoprofen–PAMAM dendrimer complex [19]

G5 PAMAM dendrimer was synthesized according to the references [20]. ketoprofen was dissolved in methanol and then the synthesized G5 PAMAM dendrimer was added. The solution was stirred overnight, then dried under vacuum in order to remove methanol. Deionized water was added to the obtained solution. The system was stirred for another 12 hours to extract the ketoprofen–PAMAM dendrimer complex. The solution was then filtered through a membrane of pore size 0.22 μm, and then lyophilized to remove water completely. The ketoprofen–PAMAM dendrimer complex was then dissolved in distilled water and the concentration of ketoprofen was characterized using a spectrophotometer at its characteristic wavelength (260 nm).

2.3. In vitro release studies

In vitro release behavior of ketoprofen from the ketoprofen–PAMAM dendrimer complex was investigated [21]. Pure ketoprofen was dissolved in methanol (2 mg/ml) and used as control. The prepared complex was dissolved in distilled water at a concentration of 2 mg/ml (the same concentration of ketoprofen as 2 mg/ml pure drug solution). This solution (2 ml in volume) was transferred to a dialysis bag (size cut off = 2.5 nm) immediately. The dialysis bag was placed in a 50 ml-beaker containing 40 ml distilled water. The outer phase was stirred continuously. After a scheduled interval of time for 12 hours, 100 μl of sample was withdrawn from the outer phase, and the outer phase was again replenished with 100 μl distilled water. The absorbance of the outer phase was monitored at 260 nm using a spectrophotometer in order to characterize the concentration of ketoprofen.

2.4. Drugs and administration

Pure ketoprofen solutions of for in vivo studies were freshly prepared by dissolving it in edible oil (2 mg/ml), while ketoprofen–PAMAM dendrimer complex was dissolved in distilled water (2 mg/ml as above) and administered orally 10 mg/kg body weights before anti-nociceptive studies and blood level studies. All the drugs were freshly administrated as prepared.

2.5. Pharmacodynamic studies

Kunming mice (20 ± 2 g each) were selected without distinction of sex. The animals were kept in well-spaced ventilated cages and maintained on healthy for 16 hours and fixed diets prior to the studies. The cages were placed in a quiet, temperature and humidity-controlled room (23 ± 1 °C and 45 ± 5%) in which a 12/12 h light–dark cycle was maintained. Anti-nociceptive was assessed by using the acetic acid-induced writhing model [22–24]. Briefly, the animals were divided into three groups with five mice each. The first group was administered no drugs and was used as a negative control; the second group was given pure ketoprofen; while the remaining group was treated with the ketoprofen–dendrimer complex. Oral administration of drugs to mice were done at scheduled intervals for 12 hours before testing. Then the mice were injected intraperitoneally with 0.6% acetic acid at a dose of 10 ml/kg body weight while manually restrained. After the injection of acetic acid, the number of writhing (stretch) responses was counted for 15 min. Each animal was used only once, and the animals were killed immediately after the termination of recording period.

2.6. Blood-level studies

Kunming mice of similar weights (20 ± 2 g) were selected without distinction of sex for blood-level studies. For each time point, two groups of mice were used, with at least four mice for each group. One was orally given pure ketoprofen, while the other group was orally administered with ketoprofen–dendrimer complex. At the scheduled time, 0.5 ml blood was taken from the ophthalmic vein of the sufficiently anesthetized animal (anesthetized with 1 g/kg of urethane). Each animal was used for once only, and the animals were killed immediately after the blood was taken. 0.2 ml of the plasma was taken after centrifugation of the heparinized blood at 10,000 rpm for 3 min and mixed with 0.5 ml methanol. After agitation for 2 min, 0.02 ml of the supernatant was taken for HPLC.

2.7. HPLC analysis

The amount of ketoprofen in the samples obtained by in vitro and blood-level studies was estimated by reversed phase high performance liquid chromatography (RT-HPLC) (Waters600, U.S.A.) [25]. The analysis was performed at 258 nm with a reversed phase C_{18} column (5 μ, 250 × 5 mm) maintained at room temperature using a mobile phase of acetonitrile: KH_2PO_4 buffer (0.025 M, pH = 3.5): double-distilled water = 49:8:43 delivered at a flow rate of 1.0 ml/min. The retention time of ketoprofen was 7.9 ± 0.1 min and the calibration curve was linear over the concentration range of 0.375–25 μg/ml ($R^2 = 0.993$).

2.8. Statistical analyses

Behavioral and pharmacokinetic data were expressed as mean ± S.E.M. and analyzed by two-tailed unpaired student's *t*-tests.

3. Results and discussion

3.1. In vitro release behavior

After the ketoprofen–PAMAM dendrimer complex was prepared. UV-Vis absorbance measurements were carried out for the characterization of ketoprofen concentration in the complex solution. The in vitro release behavior of ketoprofen from the ketoprofen–PAMAM dendrimer complex was examined in distilled water at room temperature [21]. The results are shown in Fig. 1. After two hours, 66% of the pure drug was released, whereas only 33% was released from the ketoprofen–dendrimer complex; Ten hours after the system started, 76% was released for the pure drug while 58% was released for G5 PAMAM–ketoprofen complex. The release of ketoprofen from the drug–dendrimer complex was appreciably slower compared to pure ketoprofen.

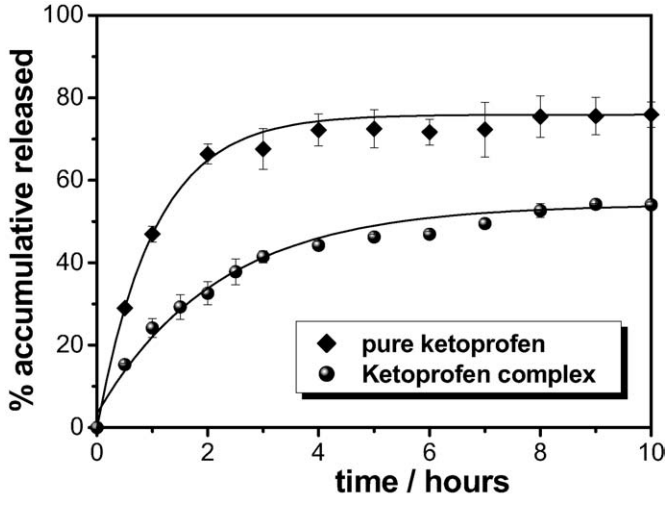

Fig. 1. In vitro release of ketoprofen from the ketoprofen–PAMAM dendrimer complex compared with the pure ketoprofen release behavior. Mean ± S.E.M.

3.2. Pharmacodynamic studies

In this study we used the acetic acid-induced writhing model to assess the anti-nociception effect of drugs. Intraperitoneal administration of a solution of 0.6% acetic acid induced a muscular constriction causing a concavity of the abdominal flank, and although comparatively rare, the behavior was easily recognized. All the drug-treated animals as well as control looked normal during the whole period of experimentation. As shown in Fig. 2, the mean number of writhing induced by the administration of acidic acid in the control mice was 27.4. Administration of pure ketoprofen significantly reduced the number of writhing, with the maximum response observed at 0.5 h post administration (mean = 1.6, P = 7.76338E – 5 compared to the control mice). The anti-nociception effect continued to 2 hours post administration. In contrast, for the groups of mice treated with ketoprofen–PAMAM dendrimer complex (equal amount of ketoprofen to pure drug groups), the maximum response was observed at 3 h (mean = 0.4, P = 3.87593E – 5 compared to the control mice), with significant anti-nociception effect lasted for 6 hours (mean = 10.4, P = 0.00456 compared to the control mice). These results showed a prolonged pharmacodynamic behavior for the ketoprofen–PAMAM dendrimer complex, which is expected to be a novel formation of NSAIDs.

3.3. Pharmacokinetic studies

The pure ketoprofen dissolved in edible oil and the complexes dissolved in distilled water were given orally to the mice. The concentration of ketoprofen in plasma was estimated at different time intervals. The blood drug concentration profiles of pure drug and the ketoprofen–PAMAM dendrimer complex are presented in Fig. 3. *The release rate of ketoprofen in the blood appears to be higher than the release rate in vitro. This may be contributed to the degradation of ketoprofen–dendrimer complex in the blood, or more particularly in the sto-*

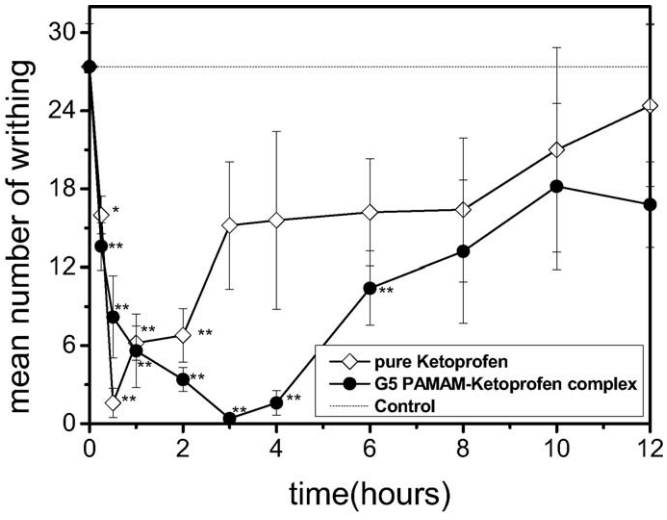

Fig. 2. Anti-nociceptive pharmcodynamic of pure ketoprofen and ketoprofen–PAMAM dendrimer complex in an acetic acid-induced writhing model. Mean ± S.E.M. (*$P < 0.05$; **$P < 0.01$, compared to the control).

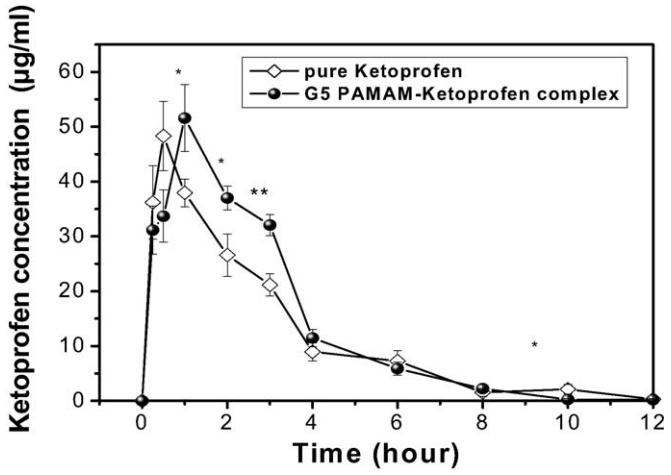

Fig. 3. Plasma ketoprofen concentration in pure ketoprofen treated animals and ketoprofen–PAMAM dendrimer complex treated animals. Mean ± S.E.M. (*$P < 0.05$; **$P < 0.01$, compared to the pure ketoprofen).

mach, as we know the complex is not stable in the acidic conditions. Maximum plasma ketoprofen concentration in ketoprofen–PAMAM dendrimer complex treated animals was attained after 1 h compared with 0.5 h for the pure ketoprofen treated ones. Higher plasma ketoprofen concentrations were observed in mice treated with ketoprofen–dendrimer complex than those treated with pure ketoprofen from 1–4 h during the experiment period, indicating a sustained release (Table 1). The C_{max} of pure ketoprofen treated animals was 48.31 μg/ml and was attained at 0.5 h with an $(AUC)_{0-12}$ of 137.23 μg/ml/h. Whereas C_{max} of the complex treat animal was 51.58 μg/ml, attained in 1 h with an $(AUC)_{0-12}$ of 160.96 μg/ml/h, respectively. In conclusion, the in vivo evaluation study indicated PAMAM dendrimers might be considered as a potential drug carrier of ketoprofen with a sustained release behavior under suitable conditions. It is interesting that the plasma ketoprofen concentration after 4 h was not in accordance with the pharmacodynamic studies; finding out the precise reason for this result will require further investigation.

4. Conclusion

Although dendrimer drug-delivery is in its infancy, it offers several attractive features. It provides a uniform platform for drug attachment that has the ability to bind and release drugs through several mechanisms [11]. *Our work demonstrated that encapsulation/interaction of ketoprofen into/with dendrimers led to sustained release of the drug both in vitro and vivo and an extended pharmacological response in vivo. We are in the process of conducting pre-clinical testing to evaluate the potential of dendrimers as carrier for ketoprofen and other NSAIDs*. Although toxicity problems may exist, modification of the structure of dendrimers should resolve this issue.

Table 1
Pharmcokinetic parameters of pure ketoprofen and ketoprofen–dendrimer complex after oral administration in Kunming mice

Parameters	t_{max} (h)	C_{max} (μg/ml)	$[AUC]_{0-12\ h}$ (μg/ml/h)
Pure ketoprofen	0.5	48.31	137.23
Ketoprofen–dendrimer complex	1	51.58	160.96

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