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Research Article

Comparison of the Analgesic Effect of Diclofenac Sodium-Eudragit® RS100 Solid Dispersion and Nanoparticles Using Formalin Test in the Rats

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Abstract

Purpose: In this study the intensity and duration of analgesic effect of diclofenac Na - Eudragit® RS100 solid dispersion and nanoparticles were evaluated by using formalin test in the rats.

Methods: The animals received different formulations of diclofenac Na and subsequently 50 µl of formalin solution (2.5%) was injected subcutaneously in the right paws after 1 h, 2 h and 3 h. The paw licking behavior was then evaluated in two phases. A dose of 20 mg/kg of pure diclofenac Na powder was determined as effective dose.

Results: In the first phase, in term of reduced paw licking time, no significant differences were found in any of the groups compared to the control group. However, in the second phase, the animals which received pure drug powder and the physical mixture of diclofenac Na with Eudragit® RS100 showed significant differences at the first and second hours. In the animals received the nanoparticles and solid dispersion, significant differences were observed in the third hour compared to the control group.

Conclusion: The analgesic effect of diclofenac Na could be improved by formulating its nanoparticles and solid dispersion with Eudragit® RS100. However, the nanoparticles revealed significantly higher analgesic effect than solid dispersion.

Introduction

Oral route is the most preferred route for drug administration due to greater convenience, less pain, high patient compliance and so on. However, oral drug delivery possesses some disadvantages like low drug solubility, poor gastrointestinal (GI) absorption, rapid metabolism, high fluctuation in the drug plasma concentration and variability in absorption due to food effects. Drug absorption from the intestine is the combination of some steps, including drug dissolution in the GI tract, uptake through the intestinal mucosa, and delivery into the systemic circulation.[1-3] Diclofenac Na is one of the most widely prescribed non-steroidal anti-inflammatory drug (NSAIDs) due to its analgesic and anti-inflammatory effects. It is administrated to relieve pain and inflammation in a wide range of musculoskeletal conditions, including various forms of arthritis, gout, back pain, dislocations, tendinitis and frozen shoulder.[4-6] Similar to other NSAIDs, diclofenac Na is associated with rare, but serious and sometimes fatal; GI tract side effects such as ulceration and hemorrhage. Therefore, this drug is an ideal candidate for taking with food and incorporation with different macromolecules to diminish its adverse effects after oral administration and also increase its bioavailability.[7-10] Eudragit® RS100, is a copolymer of poly (ethylacrylate, methylmethacrylate and chlorotrimethyl-ammonioethyl methacrylate) and contains 4.5–6.8% of quaternary ammonium groups which provide positive surface charge to the polymer. This property may cause to interact with negatively charged drugs or cellular surface of the target tissues.[10]

Solid dispersions and nanoparticles have frequently applied in order to improve physicochemical characteristics of the drugs as well as increase or decrease the drug release rate.[11-17] Our previously published study was revealed that both solid dispersions and nanoparticles of diclofenac Na – Eudragit® RS100 could considerably decrease the drug release rate.[10] In this study the solid dispersion and nanoparticles of diclofenac Na - Eudragit® RS100 with the drug: polymer ratio of 1:1 was prepared based on our previously published paper.

Subsequently, the intensity and duration of analgesic effect of the solid dispersion and nanoparticles were evaluated employing formalin test as an experimental model in male rats.

Materials and Methods

Materials

Acetone, ethanol, methanol, KH_2PO_4 and anhydrous sodium hydroxide were purchased from Merck Company (Germany). Diclofenac sodium was obtained from

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Shasun Chemicals and Drugs Company (India), Eudragit® RS100 was supplied from Boehringer Ingelheim Company (Germany).

Preparations of the nanoparticles, solid dispersion and physical mixture

Nanoparticles of diclofenac Na-Eudragit® RS100 were prepared via quasi-emulsion solvent diffusion method. Briefly, diclofenac Na and Eudragit® RS100 with the drug: polymer ratio of 1:1 were dissolved in 2 ml of ethanol at room temperature so that the total amount of drug and polymer was 100 mg. To evaporate the organic phase, the solution was stirred slowly in a shaker (MKV Orbital shaker Lh fermentation) at room temperature for 24 hrs. In order to separate the nanoparticles, the obtained suspension was centrifuged at 5 °C for 30 minutes in the 14000 rpm (Bekman: USA). The obtained nanoparticles were then lyophilized.

Coevaporation method was employed to prepare the solid dispersions of diclofenac Na-Eudragit® RS100. To this end, drug and polymer at the ratio of 1:1 with a total weight of 2 g was dissolved in 200 mL ethanol. To obtain the solid dispersions, ethanol was evaporated (Buchi® rotary evaporator, Germany) at 10 °C and 80 mmHg for an hour.

Physical mixture of diclofenac Na with Eudragit® RS100 was prepared by tumbling method (10 min) with the same drug: carrier ratio.

Animals and experimental protocol

Male Wistar rats (200 ± 20 g) were housed in the Animal House of Tabriz University of Medical Sciences at constant temperature (23 ± 2 °C) and relative humidity of 50 ± 10 % in standard polypropylene cages (four per cage), under a 12/12 hrs light/dark cycle. Animals were freely allowed food and water. This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Tabriz University of Medical Sciences, Tabriz-Iran (National Institutes of Health publication No 85–23, revised 1985).

The rats were divided into a control group and 18 test groups, which consisted of 8 rats in each group. The animals were fed with standard laboratory food (rat chow) for one week.

To check the presence of any possible analgesic effect of normal saline, 2 mL of normal saline solution was gavaged in one group. In the control group only 50 µl of formalin solution 2.5 % (v: v) was injected subcutaneously into right foot of the animals and the rats did not receive any drug.

In the test groups, formulations were dispersed in 2 mL of normal saline solution and then were gavaged orally according to the following protocol: Four groups were received pure diclofenac Na powder (2.5, 5, 10, and 20 mg/Kg); three groups were received an effective dose of pure diclofenac Na powder (20 mg/Kg); three groups were received the nanoformulation; three groups were received the solid dispersion; and finally three groups were received the physical mixture (all equivalent to effective dose). All tests were performed between 8 am to 2 pm.

One hour (in the first group), two hours (in the second group) and three hours (in the third group) after the gavage, formalin solution was injected subcutaneously in the right foot. Immediately after injection, the animals were placed in the special glass chamber (20×25×40 cm) equipped with a mirror (with a 45 degree angle in the bottom) to evaluate the reaction to pain carefully. Reactions to injection of formalin solution was measured by stopwatch and recorded up to 5 minutes, in the first phase, and from 20 to 60 minutes, in the second phase.

Statistical analysis

One way ANOVA test followed by Tukey post test was used for comparisons between the treatment and control groups. Data were presented as Mean ± SEM. The P values <0.05 was considered as significance level during this study.

Results

Effect of different doses of formalin on licking time

In the animals which received formalin 2.5 % (v:v), paw licking time in the phase I (0-5 minutes) and phase II (20-60 min) were 79.0 ± 5.4 and 260.1 ± 6.7 seconds, respectively. Subcutaneous injection of 50 µl formalin solution 5 % (v:v) did not show any significant difference (p>0.05) in the duration of pain when compared with formalin 2.5% (Figure 1). Therefore, in order to avoid complications due to increased concentrations of formalin and tissue destruction, formalin 2.5 % (v: v) was applied to induce the inflammatory pain.

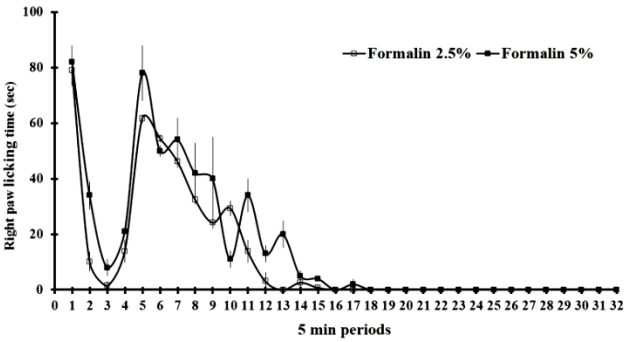

Figure 1. Effect of different doses of formalin on the licking time in the rats

Effect of different doses of pure diclofenac Na powder on the licking time

In order to investigate the effect of different doses of pure diclofenac Na on the licking time as well as to find the effective dose of the drug, four doses of pure drug powder (2.5, 5, 10, and 20 mg/Kg) were administered to the four groups of animals one hour before the injection of formalin.

In the first phase, no significant difference (p>0.05) was detected between the control group and the groups

which received different doses of pure diclofenac Na. However, in the second phase, only the group which received a dose of 20 mg/kg of pure drug powder showed significant difference ($p<0.05$) in the licking time (220.25 ± 8.0 sec) compared to the control group (268.6 ± 9.4 sec) (Table 1). Therefore, the effective dose of pure diclofenac Na powder was determined as 20 mg/kg.

Table 1. Effect of different doses of pure diclofenac on licking time in phases I and II.

Group	Phase I (sec)	Phase II (sec)
Control	79.0 ± 5.4	268.6 ± 9.4
Normal Saline	81.0 ± 3.6	260.5± 8.9
Diclofenac 2.5 mg	74.8 ± 5.0	260.1±6.7
Diclofenac 5 mg	74.5 ± 3.2	251.2 ± 7.3
Diclofenac 10 mg	70.6 ± 6.1	246.3 ± 8.1
Diclofenac 20 mg	79.0 ± 5.4	220.2 ±8.1*

Results were expressed as mean ± SEM. (*: $p<0.05$ compared to the control group).

The animals which received 20 mg/mL of pure diclofenac Na powder did not show any significant difference ($p>0.05$) in phase I. On the other hand, following administration of the pure drug after one and two hours (phase II) the licking time was decreased significantly ($p<0.05$) from 268.6 ± 9.4 sec to 220.2 ± 8.0 sec and 218.8 ± 13.5 sec, respectively. However, after 3 h, decrease in licking time was not significant ($p>0.05$) (Table 2).

Table 2. Effect of different formulations on licking time in phases I and II at times 1h, 2h and 3h.

Group		Phase I (sec)	Phase II (sec)
Control		79.0 ±5.4	268.6 ±9.4
Pure Diclofenac	**1 h**	70.6 ±6.2	220.2 ±8.0*
	2 h	74.1 ±7.1	218.8 ±13.5*
	3 h	76.5 ±5.4	240.7 ±9.4
Physical mixture	**1 h**	75.0 ±8.6	224.2 ± 13.5*
	2 h	75.2 ±4.2	223.0 ±11.1*
	3 h	81.6 ±6.9	243.0 ±10.4
Solid dispersion	**1 h**	73.2 ±8.1	190.1 ±7.7***
	2 h	76.5 ±5.2	189.1 ±9.3***,#
	3 h	76.6 ±7.8	192.5 ±7.9***,##
Nanoparticles	**1 h**	70.0 ±5.9	166.7± 7.1***,a
	2 h	71.2 ±7.3	165.6 ±8.2***,###,a
	3 h	72.6 ±5.9	167.6 ±6.7***,###,a

Results were expressed as mean ±SEM. (*: $p<0.05$ and ***: $p< 0.001$ compared to the control group,#: $p<0.05$, ##: $p<0.01$ and ###: $p< 0.001$ compared to the pure diclofenac received groupand[a]: $p<0.05$) compared to the solid dispersionreceived group.

Effect of physical mixtures of diclofenac Na and Eudragit® RS100 on the licking time

In the phase I, there was no significant difference ($p>0.05$) between the control group and the group which received the physical mixture (Table 2). Nevertheless, the significant difference ($p<0.05$) was revealed between these groups only in times 1 h and 2 h of the phase II. However, the effects of physical mixtures did not significantly differ ($p>0.05$) from that of pure drug powder in the corresponding times.

Effect of the solid dispersion on licking time in phase I and II after different times

Similar to the previous groups, in the phase I, the licking times in the group which received the solid dispersion were not significantly ($p>0.05$) decreased compared to the control group (Table 2). However, in the phase II, difference in the licking time after all the three times was remarkably significant ($p<0.001$) compared to the control group. Then again, in comparison with pure diclofenac Na powder, the solid dispersion could significantly decrease the licking time after 2 h ($p<0.05$) and 3 h ($p<0.01$).

Effect of the nanoparticles on licking time

As it is shown in Table 2, the nanoparticles of diclofenac Na-Eudragit® RS100 could not decrease the licking time in phase I. However, in phase II, in all of the administered times, a significant difference was observed between the group which received the nanoparticles and the control group ($p<0.001$). Furthermore, the licking time in the nanoparticle administered animals was significantly less than the group which received pure drug powder ($p<0.001$) and the solid dispersion ($p<0.05$).

Discussion

The analgesic effects of pure diclofenac Na powder in the consecutive hours of pain are shown in Table 1. In the first phase, there was no significant difference ($p>0.05$) between pure diclofenac Na administered group and the control group demonstrating that diclofenac Na was not effective in the acute phase of pain. However, the analgesic effect was detected in the chronic phase of the pain. Lack of significant differences in the third hours of second phase, could be due to low half-life of drug in plasma (1-2 hr).

The analgesic effect of the physical mixture was not significantly ($p>0.05$) higher than that of pure drug powder, signifying that Eudragit® RS100 had no effect on pain reduction nevertheless the process and the type of formulation was important.

Table 2 also reveals that the analgesic effects of the solid dispersion and nanoparticles did not also show significant differences ($p>0.05$) in the first phase compared to the control group. However, the analgesic differences at the third hour (chronic phase) were noticeably significant ($p<0.05$) for the both formulations compared to the control group. Moreover, the results showed that these formulations had retained their

analgesic effect which could be because of the slow release of diclofenac Na in the GI environments.

In the first phase, there was no significant difference ($p>0.05$) between the analgesic effect of the nanoparticles and solid dispersion, nevertheless the appropriate analgesic effect was observed by the nanoparticles in the chronic phase. This effect was interestingly superior than the solid dispersion associated analgesic effect which could be owing to the slow release of the drug from the nanoparticles as well as the increased absorption of the nanoparticles.

The comparison of the analgesic effects of the different formulations in the third hour after the pain experience indicated that unlike the pure drug powder and the physical mixture, the solid dispersion and nanoparticles prolonged the analgesic effect which could be as a result of slow drug release from these formulations.

According to our previously published findings, the mean particle size of diclofenac Na-Eudragit® RS100 nanoparticles with the drug/polymer ratio of 1:1 was 103±60 nm and the size distribution was relatively monodisperse (polydispersity index = 0.320 ± 0.083).[10] Due to sub-micron size of the nanoparticles, these particles can easily penetrate into the tissue, which lead to the reaching of the appropriate amount of drug into the targeted site of the body.[18] Other features of the nanoparticles are the delivery of drugs into the specific parts or organelles inside the cells making possible the increased bioavailability and reduced side effects of the drugs.[18]

Uptake of the particles can be take place by various processes such as phagocytosis, endocytosis or pinocytosis which are receptor-mediated.[19,20] Eudragit® RS100 with the zeta potential of 35 mV, produces positive charge on the surface of the nanoparticles and facilitate their uptake by the negatively charged cells.

Conclusion

According to the formalin test results, the nanoparticles and solid dispersion of diclofenac Na and Eudragit® RS100 showed higher intense and also duration of analgesic effects than pure drug which could be attributed to the improved physicochemical property and sustained release of the drug from these formulations. Physical mixture of diclofenac Na and Eudragit® RS100 showed similar analgesic effects with the pure drug powder. Nanoparticles also showed better analgesic effect than the solid dispersion which can be due to the higher absorption of the nanoparticles from GI tract and/or reduced drug removal by the phagocytic system. Because of the high risk of diclofenac Na toxicity which can initiate severe side effects in gastrointestinal track such as peptic ulceration and gastrointestinal bleeding, nanoparticles and solid dispersions of diclofenac Na could be considered as suitable candidates for drug delivery.

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Ethical Issues

Not applicable.

Conflict of Interest

The authors declare no conflict of interests.

References

1. Di Maio S, Carrier RL. Gastrointestinal contents in fasted state and post-lipid ingestion: In vivo measurements and in vitro models for studying oral drug delivery. *J Control Release* 2011;151(2):110-22.
2. Keller J. Gastrointestinal Digestion and Absorption. In: Lennarz WJ, Lane MD, editors. Encyclopedia of Biological Chemistry. Waltham: Academic Press; 2013. P. 354-9.
3. Tao SL, Desai TA. Gastrointestinal patch systems for oral drug delivery. *Drug Discov Today* 2005;10(13):909-15.
4. Baraf HS, Gold MS, Petruschke RA, Wieman MS. Tolerability of topical diclofenac sodium 1% gel for osteoarthritis in seniors and patients with comorbidities. *Am J Geriatr Pharmacother* 2012;10(1):47-60.
5. De La Garza RII, Fabrizio KR, Radoi GE, Vlad T, Asnis GM. The non-steroidal anti-inflammatory drug diclofenac sodium attenuates lipopolysaccharide-induced alterations to reward behavior and corticosterone release. *Behav Brain Res* 2004;149(1):77-85.
6. Piao H, Kamiya N, Watanabe J, Yokoyama H, Hirata A, Fujii T, et al. Oral delivery of diclofenac sodium using a novel solid-in-oil suspension. *Int J Pharm* 2006;313(1–2):159-62.
7. Tawfeek HM. Evaluation of PEG and mPEG-co-(PGA-co-PDL) microparticles loaded with sodium diclofenac. *Saudi Pharm J* 2013;21(4):387-97.
8. Nayak AK, Pal D. Development of pH-sensitive tamarind seed polysaccharide-alginate composite beads for controlled diclofenac sodium delivery using response surface methodology. *Int J Biol Macromol* 2011;49(4):784-93.
9. Soares GA, De Castro AD, Cury BS, Evangelista RC. Blends of cross-linked high amylose starch/pectin loaded with diclofenac. *Carbohydr Polym* 2013;91(1):135-42.
10. Barzegar-Jalali M, Alaei-Beirami M, Javadzadeh Y, Mohammadi G, Hamidi A, Andalib S, et al. Comparison of physicochemical characteristics and drug release of diclofenac sodium–eudragit® RS100 nanoparticles and solid dispersions. *Powder Technol* 2012;219:211-6.

11. Adibkia K, Barzegar-Jalali M, Javadzadeh Y, Maheri-Esfanjani H. A review on the methods and applications of spray drying technology. *Pharmaceutical Sciences* 2012;18(2):119-32.
12. Adibkia K, Barzegar-Jalali M, Maheri-Esfanjani H, Ghanbarzadeh S, Shokri J, Sabzevari A, et al. Physicochemical characterization of naproxen solid dispersions prepared via spray drying technology. *Powder Technol* 2013;246:448-55.
13. Adibkia K, Barzegar-Jalali M, Mohammadi G, Ebrahimnejhad H, Alaei-Beirami M. Effect of sodium alginate chain length and Ca2+and Al 3+on the release of diltiazem from matrices. *Pharmaceutical Sciences* 2011;16(4):221-8.
14. Adibkia K, Barzegar-Jalali M, Nokhodchi A, Siahi Shadbad MR, Omidi Y, Javadzadeh Y, et al. A review on the methods of preparation of pharmaceutical nanoparticles. *Pharmaceutical Sciences* 2009;15(4):303-14.
15. Adibkia K, Javadzadeh Y, Dastmalchi S, Mohammadi G, Niri FK, Alaei-Beirami M. Naproxen-eudragit® RS100 nanoparticles: Preparation and physicochemical characterization. *Colloids Surf B Biointerfaces* 2011;83(1):155-9.
16. Barzegar-Jalali M, Dastmalchi S. Kinetic analysis of chlorpropamide dissolution from solid dispersions. *Drug Dev Ind Pharm* 2007;33(1):63-70.
17. Javadzadeh Y, Adibkia K, Bozorgmehr Z, Dastmalchi S. Evaluating retardation and physicochemical properties of co-ground mixture of Na- diclofenac with magnesium stearate Original Research Article. *Powder Technol* 2012;218:51-6.
18. Panyam J, Labhasetwar V. Biodegradable nanoparticles for drug and gene delivery to cells and tissue. *Adv Drug Deliv Rev* 2002;55(3):329-47.
19. Foster KA, Yazdanin M, Audus KL. Microparticulate uptake mechanisms of in-vitro cell culture models of the respiratory epithelium. *J Pharm Pharmacol* 2001;53(1):57-66.
20. Suh H, Jeong B, Rathi R, Kim SW. Regulation of smooth muscle cell proliferation using paclitaxel-loaded poly(ethylene oxide)-poly(lactide/glycolide) nanospheres. *J Biomed Mater Res* 1998;42(2):331-8.