Drug Development and Industrial Pharmacy, 2009; 35(9): 1043–1047

informa
healthcare

ORIGINAL ARTICLE

Pharmacokinetics of intramuscular microparticle depot of valdecoxib in an experimental model

Sagar M. Agnihotri and Pradeep R. Vavia

Pharmaceutical Division, Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai, India

Abstract

Aim: We did a prospective study to investigate pharmacokinetics of a single intramuscularly (i.m.) administered Valdecoxib (VC) polymeric microparticles in New Zealand white rabbits. *Method*: Poly[lac(glc-leu)] microparticles encapsulating a potent cyclooxygenase-2- selective inhibitor, VC, were prepared by emulsion and solvent evaporation technique and administered i.m. to rabbits for pharmacokinetic study. *Results*: A single i.m. dose of drug-loaded poly[lac(glc-leu)] microparticles resulted in sustained therapeutic drug levels in the plasma for 49 days. The relative bioavailability was increased severalfold as compared with unencapsulated drug. *Conclusions*: Injectable poly[lac(glc-leu)] microparticles hold promise for increasing drug bioavailability and reducing dosing frequency for better management of rheumatoid arthritis.

Key words: *Bioavailability; depot formulation; pharmacokinetics; poly[lac(glc-leu)]; valdecoxib*

Introduction

Valdecoxib (VC), 4-(5-methyl-3-phenylisoxazol-4-yl)benzenesulfonamide, is an anti-inflammatory drug that is highly selective for inhibition of the inducible form of cyclooxygenase (COX-2). COX-2 is induced by mediators of inflammation and is active in mediating inflammation and pain. COX-2 also plays physiological roles in a limited number of tissues, including those of the female reproductive tract, the kidney, and possibly the vascular endothelium. The other isoform of COX, that is, COX-1, exists constitutively in most tissues, including the gastrointestinal (GI) tract, kidney, and platelets. The prostaglandins produced by COX-1 play a key role in the maintenance of physiological functions such as platelet aggregation and are among the factors that maintain the GI mucosal barrier. At therapeutic plasma concentrations in humans, VC does not inhibit COX-1. This drug (Bextra; Pharmacia Corp., New York, USA) was approved by the US Food and Drug Administration for the treatment of rheumatoid arthritis (RA), osteoarthritis, and primary dysmenorrhea in November 2001[1,2]. However, on April 7, 2005, Pfizer withdrew Bextra from the US market on recommendation by the FDA because of an increased risk for heart attacks and stroke and also the risk of a serious, sometimes fatal, skin reaction.

VC is the second-generation COX-2 inhibitor developed by Pharmacia after celecoxib, the first approved COX-2 inhibitor. These new types of anti-inflammatory drugs are developed based on the hypothesis that selective inhibition of COX-2 should decrease inflammation without the adverse GI effects associated with inhibition of the constitutive cyclooxygenase (COX-1)[3–5]. Clinical studies have demonstrated that COX-2 inhibitors lead to a significant reduction in joint pain, joint tenderness/pain, and joint swelling with a statistically significantly lower incidence of gastric ulceration[6,7].

In the treatment of RA, the fact that a RA patient needs to take anti-inflammatory drugs daily is largely responsible for patient noncompliance and therapeutic failure. The development of a controlled-release formulation is a possible solution to this problem[8–10]. The side effects of anti-inflammatory drugs, such as ulceration of the kidney and central nervous system toxicity, limit their use for RA. Encapsulation of such drugs in microparticle (MP) or liposomal formulations may reduce the toxic effects, target the drug to specific site, and could improve bioavailability[11–13]. Here, we

Address for correspondence: Sagar M. Agnihotri, PhD, Pharmaceutical Division, Institute of Chemical Technology, University of Mumbai, Nathalal Parikh Street, Matunga, Mumbai 400 019, India. E-mail: agnihotrisagar@hotmail.com

(Received 9 Sep 2008; accepted 19 Jan 2009)

ISSN 0363-9045 print/ISSN 1520-5762 online © Informa UK, Ltd.
DOI: 10.1080/03639040902762979

report on the pharmacokinetics of a single i.m. administration of VC polymeric MPs in New Zealand white rabbit model.

Materials and methods

Materials

VC was a generous gift from Glenmark Pharmaceuticals (Mumbai, India), polyvinyl alcohol was gifted by Colorcon (Mumbai, India), poly[lactic acid(glycolic acid-leucine] (poly[lac(glc-leu)]) (Mw = 7474 Da) was used as synthesized in our laboratory[14]; all other chemicals and reagents were of analytical grade and available commercially. HPLC system with multiwavelength detector was from Jasco (Tokyo, Japan).

Preparation of microparticles

VC polymeric microparticles (VC-MP) were prepared by the o/w emulsion and solvent evaporation method[15]. Briefly, VC (50 mg) and poly[Lac(Glc-Leu)] (250 mg) were dissolved in 5 mL methylene chloride, this solution was then slowly injected with a syringe of 20 gauze needle connected to a thin teflon tube, into 125 mL water containing polyvinyl alcohol (0.4%, w/v). During injection, the mixture was highly mixed at a agitation speed of 2000 rpm. The resulting emulsion obtained was sonicated in probe-type sonicator and then stirring was continued for 60 minutes. Solvent residues were left to evaporate off under a slow magnetic stirring at room temperature for 8–12 hours. The suspension was freeze-dried (Labconco lyophilizer, Labconco Corporation, Kansas City, MO, USA) for 24 hours at –40°C and at a pressure of 0.05 mm Hg.

Microparticle characterization

The mean particle size of the formulation was determined by particle size analyzer (Beckman Coulter N+ Plus; Wipro, India Ltd., Mumbai, India) equipped with software N4 Plus. The scanning electron micrograph (SEM) of the VC-MP preparation was taken with a Jeol JSM-6400 electron microscope (Philips, Tokyo, Japan). For drug content analysis of MP formulation, the MP (10 mg) was accurately weighed and dissolved in 2 mL dichloromethane. The polymer was precipitated using methanol (volume up to 10 mL). After centrifugation at 5000 × *g* for 5 minutes, the clear supernatant obtained was analyzed by HPLC, and the VC concentration encapsulated in MPs was measured. The stability of the prepared VC-MP was monitored by the determination of the particle size, homogenicity, and drug content. The MPs were monitored immediately after preparation and periodically over a year at 4°C–6°C (refrigerator) or at 25°C/60% RH away from direct light.

Animal experiments

New Zealand albino rabbits, weighing 1.8–2.2 kg, were used in the study. The study was performed in accordance with the Institutional Animal Ethics Committee (IAEC) constituted as per directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under the Ministry of animal welfare division, Government of India, New Delhi. The animals were housed individually in standard cages for 12 hours in light–dark cycle and were given free access to food and water. Each rabbit was kept in a metabolic cage in an animal room for at least 1 week before the study. One hour prior to the experiment, each rabbit was held in a restraining device, the hair in the ears was shaved with a razor blade. The animals were housed into four groups, six rabbits in each group.

Group I: VC plain drug (oral route, 10 mg)
Group II: VC plain drug (oral route, 30 mg)
Group III: VC plain drug (i.m. injection, 30 mg)
Group IV: VC-MP preparation (equivalent to 30 mg VC)

The plain drug for oral dose was suspended in 1% (w/v) sodium carboxylmethyl cellulose. Each animal received formulations by the oral route with a gastric catheter, which was subsequently flushed with 10 mL water.

For i.m. dosing, plain drug and VC-MP were suspended in saline (1 mL). The product after reconstitution was injected into New Zealand white rabbits. The injection was carried out using sterile disposable hypodermic syringe equipped with 20-gauge needle. Before injecting the product, blood samples were taken and this served as the initial 0 day reading.

Blood samples (1 mL) were drawn from the marginal vein of the ear at 0, 0.5, 1, 2, 4, 8, and 12 hours for plain drug dose and 0, 7, 14, 21, 28, 35, 42, and 49 days for the MP depot dose. After centrifugation, plasma was collected in polypropylene tubes and stored frozen at –70°C until analysis.

HPLC analysis of valdecoxib

The HPLC assay of Ramakrishna et al.[16] was used to analyze VC concentrations with some modifications. Briefly, 0.5 mL volume of protein-free rabbit plasma was transferred to a 15-mL glass test tube and then 100 µL of VC working solution (10 µg/mL) was spiked. Further, a 2 mL aliquot of extraction solvent, diethyl ether/dichloromethane (7/3), was added. The sample was vortex-mixed for 5 minutes. The sample was then centrifuged for 5 minutes at 5000 × *g*. The organic layer was quantitatively transferred to a 5-mL glass tube and evaporated to dryness under a stream of nitrogen. The dried extract was reconstituted in 500 µL of acetonitrile and a 300-µL

aliquot was injected into a C18 column (ODS, 250 mm × 4.6 mm, 5 μm; Merck, India). Detection was via VC intrinsic UV absorption at 210 nm on the HPLC system with multiwavelength detector (Jasco MD-2015 plus, Japan). The mobile phase consisted of double-distilled water (pH 3.8, 1% glacial acetic acid and 0.1% triethyl amine) : acetonitrile (55:45) delivered at a rate of 0.8 mL/minute. The limit of quantification (LOQ) was 360 ng/mL. In plasma, coefficient of variation (CV) of precision and accuracy was <4% and recovery was >85%.

Data analysis

The plasma concentration data were fitted using Basica V 3.20 pharmacokinetic software (Microsoft Corp., Redmond, WA, USA), to calculate the area under the curve (AUC) and terminal elimination half life ($T_{1/2}$). Data are presented as mean ± SD.

Results and discussion

The particle size, determined by photon correlation spectroscopy, ranged from 1123 to 2150 nm (polydispersity index: 0.173 ± 0.04). The SEM of VC-MP used for biological experiments is shown in Figure 1. The SEM picture of VC-MPs revealed smooth and spherical surface. For reproducible experiments, it is important to know the content of encapsulated VC. With the described HPLC method, the content of VC is determinable. The drug encapsulation efficiency was 67.73% ± 1.94%. The particle size and drug content data of the MP preparation used in the in vivo experiments are shown in Table 1. The VC-MP were physicochemically stable over a year (period of observation) at the selected storage conditions. Neither the mean diameter nor the drug content showed essential changes.

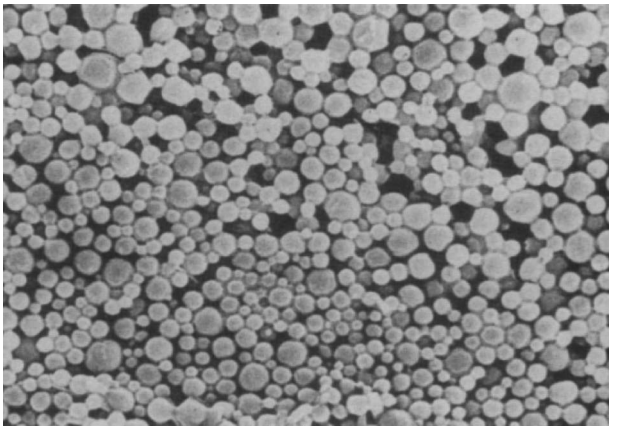

Figure 1. Electron micrograph of the VC-MP preparation.

Plasma distribution of VC is represented as the values of concentration (μg VC/mL of plasma) found for VC (oral 10, 30, and i.m. 30 mg doses) and VC-MP system (i.m. dose, equivalent to 30 mg VC). The plasma pharmacokinetics of VC is depicted in Table 2. Dose proportionality was demonstrated after single oral doses (10–30 mg) of VC. Bioavailability of VC given orally was not clinically significantly different compared to VC given intramuscularly (Figure 2). In case of plain VC, the drug disappeared from the circulating blood very rapidly because of its short half-life. The VC-MP showed significantly higher C_{max} (78.43 mcg/mL) as compared to plain VC i.m. injection (C_{max} 68.42 mcg/mL). T_{max} of plain VC i.m. injection was found to be 1 hour whereas that of VC-MP was 28 days. Delayed T_{max} of VC-MP could be attributed to sustained activity (Figure 3). VC-MP showed 160-fold increase in AUC as compared to plain VC 10 mg/30 mg oral dose and VC-MP showed 192-fold increase in AUC as compared to plain VC 30 mg i.m. dose.

No clear correlation between the pharmacokinetic parameters of i.m.-dosed VC plain drug and VC-MP could be established. This lack of clear correlation may be due in part to the fact that VC concentrations measured here reflect both free and encapsulated VC. We hypothesize that i.m.-injected MPs reach unchanged in circulation. These observations are supported by the work of Casley-Smith et al. They found that lymphatics close to the muscle have open junctions with spaces as large as 0.5–10 μm that is an indication that a portion of the i.m.-injected MPs reached unchanged in the circulation[17,18]. Furthermore, small MPs could have survived longer in the circulation[19]. Moreover, VC is 98% bound to plasma proteins, the significant difference in the relative bioavailability could be due to displacement of the drug from the plasma protein binding site. In this way, the high concentrations of VC in the plasma were obtained for the i.m.-injected VC-MP.

It is clear that a single i.m. injection of drug-loaded polymeric MPs demonstrated a better extent of absorption

Table 1. Effects of storage time and conditions on the mean size and drug content values of VC-MP.

	Mean size (nm) ± SD			Drug content (%) ± SD		
Preparation	Initial value	After 12 months at 5°C (±2°C)	After 12 months at 25°C/60% RH	Initial value	After 12 months at 5°C (±2°C)	After 12 months at 25°C/60% RH
VC-MP	1123 ± 20	1843 ± 18	2150 ± 22	67.73 ± 1.3	66.28 ± 1.7	65.69 ± 1.8

Table 2. Summary of pharmacokinetic parameters (n = 6 rabbits/group) for VC plain drug and VC-MP.

Treatment	Route	Dose equivalent to VC (mg)	C_{max} (μg/mL)	T_{max} (hours)	$T_{1/2}$ (hours)	$AUC_{0-\infty}$ (μg. hour/mL)
Plain drug	Oral	10	17.76	2	1.7	75.5
	Oral	30	62.36	2	2.36	229
	i.m.	30	68.42	1	2.8	188
VC-MP	i.m.	30	78.43	672	139	35334

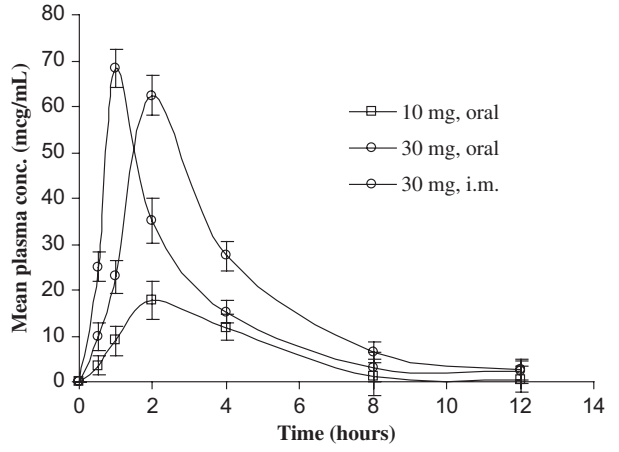

Figure 2. Plasma concentration–time profiles of administered VC (plain drug) 10 mg oral dose, 30 mg oral dose, and 30 mg i.m. dose. Data are represented as means (n = 6 rabbits/group). Error bars, SD.

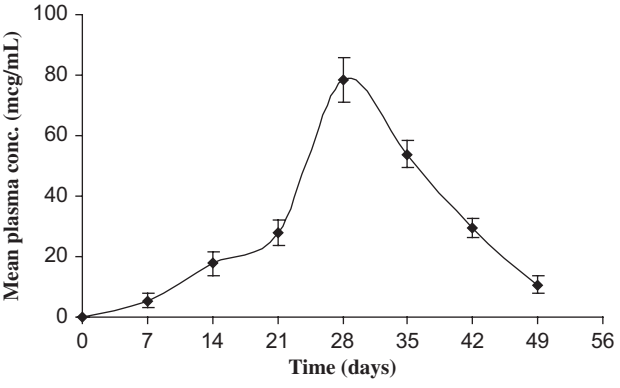

Figure 3. Plasma concentration–time profile after i.m. administration of VC-MP at VC dose of 30 mg. Data represented as mean (n = 6 rabbits). Error bars, SD.

as compared with oral and i.m. free drug. Although from the patient's point of view intramuscular dosing is not a favorite route of drug administration, it is expected that a reduction in dosing frequency from daily conventional doses to one intramuscular dose would significantly improve patient compliance and help in the better management of RA.

Conclusion

The present communication, employing intramuscular polymeric MP, suggests a reduction in dosing frequency to one and merits further evaluation in a higher animal model.

Acknowledgment

The authors express their thanks to Council of Scientific and Industrial Research (Govt. of India, New Delhi) for financial support.

Declaration of interest: The authors report no conflicts of interest.

References

1. Camu F, Beecher T, Recker DP, Verburg KM. (2002). Valdecoxib a COX-2-specific inhibitor is an efficacious opioid-sparing analgesic in patients undergoing hip arthroplasty. Am J Ther, 9:43–51.
2. Fricke J, Varkalis J, Zwillich S, Adler R, Forester E, Recker DP, et al. (2002). Valdecoxib is more efficacious than rofecoxib in relieving pain associated with oral surgery. Am J Ther, 9:89–97.
3. Donnelly MT, Hawkey CJ. (1997). COX-2 inhibitors—a new generation of safer NSAIDs. Aliment Pharmacol Ther, 11:227–36.
4. Pennisi E. (1998). Building better aspirin and does aspirin ward off cancer and Alzheimer's. Science, 280:1191–2.
5. Vane JR, Bakhle YS, (1998). Botting RM. Cyclooxygense 1 and 2. Annu Rev Pharmacol Toxicol, 38:97–120.
6. Isakson PC, Zweifel BS, Masferrer JL, Koboldt C, Seibert K, Hubbard RC, et al. (1998). Specific COX-2 inhibitors: From bench to bedside. In: Vane J, Botting J, eds. Pharmacology, clinical effects and therapeutic potential. London: Kluwer Academic Publishers and William Harvey Press, 127–33.
7. Simon LS, Lanza FL, Lipsky PE, Hubbard RC, Talwalker S, Schwartz BD et al. (1998). Preliminary study of the safety and efficacy of SC-58635 a novel cycolooxygenase 2 inhibitor. Arthritis Rheum, 41:1591–1602.
8. Tuncay M, Calis S, Kas HS, Ercan MT, Peksoy I, Hincal AA. (2000). In vitro and in vivo evaluation of diclofenac sodium loaded albumin microspheres. J Microencapsul, 17(2):145–55.
9. Tuncay M, Calis S, Kas HS, Ercan MT, Peksoy I, Hincal AA. (2000). Diclofenac sodium incorporated PLGA (50:50) microspheres: Formulation considerations and in vitro/in vivo evaluation. Int J Pharm, 195(1–2):179–88.
10. Puebla P, Pastoriza P, Barcia E, Fernandez-Carballido A. (2005). PEG-derivative effectively modifies the characteristics of indomethacin-PLGA microspheres destined to intra-articular administration. J Microencapsul, 22(7):793–808.

11. Srinath P, Chary MG, Vyas SP, Diwan PV. (2000). Preparation and pharmacodynamic evaluation of liposomes of indomethacin. Drug Dev Ind Pharm, 26(3):313–21.
12. Srinath P, Chary MG, Vyas SP, Diwan PV. (2000). Long-circulating liposomes of indomethacin in arthritic rats—a biodisposition study. Pharm Acta Helv, 74(4):399–404.
13. Rajendrakumar K, Madhusudan S, Pralhad T. (2005). Cyclodextrin complexes of valdecoxib: Properties and anti-inflammatory activity in rat. Eur J Pharm Biopharm, 60(1):39–46.
14. Agnihotri SM, Vavia PR. (2006). Effects of formulation variables on the formation of nanoparticles prepared from L-lactide-depsipeptide copolymer. J Biomed Nanotech, 2(3–4):239–44.
15. Tuncay M, Alis SC, Kas HS, Ercan MT, Peksoy I, Hincal AA. (2000). Diclofenac sodium incorporated PLGA (50:50) microspheres: Formulation considerations and in vitro: In vivo evaluation. Int J Pharm, 195:179–88.
16. Ramakrishna NVS, Vishwottam KN, Wishu S, Koteshwara M. (2004). Quantitation of valdecoxib in human plasma by high-performance liquid chromatography with ultraviolet absorbance detection using liquid-liquid extraction. J Chromatogr B, 802(2):271–5.
17. Casley-Smith JR. (1964). An electron microscopic study of injured and abnormally permeable lymphatics. Ann NY Acad Sci, 116:803–30.
18. Naeff R, Pliska V, Weder HG. (1990). Pharmacokinetics of small unilamellar liposomes and incorporated lonazolac after i.m. administration. J Microencapsulation, 7(1):95–103.
19. Pandey R, Khuller GK. (2004). Subcutaneous nanoparticle-based antitubercular chemotherapy in an experimental model. J Antimicrob Chemother, 54:266–8.