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Biodegradable Injectable Implant Systems for Long Term Drug Delivery Using Poly (Lactic-co-glycolic) Acid Copolymers

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Abstract

Poly (lactide-co-glycolide) (PLG), is one of the most widely employed biodegradable synthetic polymers for sustained-release preparations. In the present work, PLG (50:50) copolymer has been used to deliver diclofenac sodium in the form of microspheres and in situ gel-forming systems, both of which can be injected subcutaneously.

The pharmacodynamic and pharmacokinetic studies in the adjuvant-induced arthritic rats showed that the microspheres offered steady therapeutic levels of the drug in the plasma for about 16 days following a single subcutaneous injection. However, the in situ gel-forming system provided a significantly higher maximum plasma concentration and increased inhibition of inflammation, maintained for about 10 days.

Injectable microspheres and in situ gel-forming implant systems of PLG (50:50) copolymer may therefore be considered as prospective implantable controlled-release dosage forms to deliver drugs in long-term therapy of chronic ailments.

Significant research interest in the development of subcutaneously implantable polymeric devices for long-term maintenance of therapeutic drug levels coincides with the increased medical and public acceptance of such systems (Tice 1985; Langer 1990). Implantable controlled release systems provide advantages over conventional drug therapies (Danckwerts & Fassihi 1991). Their advantages and the emerging applications in controlled drug delivery have been reviewed (Leong & Langer 1987; Wise et al 1987).

Poly (lactide-co-glycolide) (PLG), a copolymer of polylactic acid and poly (glycolic acid) is the most widely used biodegradable synthetic polymer for long-term parenteral (particularly subcutaneous) delivery of different classes of drugs in the form of microspheres and nanoparticles (Jalil 1990; Brannon-Peppas 1995). Recently, biodegradable in situ gel-forming drug delivery systems have also used PLG coplymers (Shah et al 1993). These formulations swell into gel matrices on contact with aqueous fluids thereby releasing the incorporated drug slowly over a period of weeks to months. Further, as with the drug-loaded biodegradable microparticulate carriers, the gel-forming systems can be conventionally injected subcutaneously. Thus, an attempt has been made to envisage the potential advantages of injectable PLG implants for long-term delivery of diclofenac, commonly used in the therapy of rheumatoid arthritis.

Diclofenac has a mean terminal plasma half-life of 1·8 h after oral dosing. Fifty percent of orally-dosed diclofenac does not reach the systemic circulation, due predominantly, to first-pass metabolism (Willis et al 1979; Salmann 1986). As with other nonsteroidal anti-inflammatory drugs, gastrointestinal effects are the predominant adverse reactions on frequent oral administration of diclofenac (Caldwell 1986; Abakken 1992). It is also notable that diclofenac offers a prolonged clinical effect despite its short elimination half-life. This is found to be due to higher levels of diclofenac and its metabolites in the synovial fluid than in plasma, at later times after dosing (Fowler et al 1983). Consequently, maintenance of drug and active metabolites at relatively high and constant levels in the synovial fluid can enhance the drug's overall anti-inflammatory effect. Approaches involving parenteral polymeric dosage forms that would constantly release and maintain diclofenac levels in plasma over a long period of time, and which may reduce the localized topical gastroduodenal toxicity that would occur due to frequent oral administration of the drug (Hoftiezer et al 1980) have attracted interest. The present communication is a report on the results of subcutaneously implantable microspheres and in situ gel-forming preparations prepared using PLG copolymers.

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Materials and Methods

Diclofenac sodium was obtained from Bangalore Pharmaceutical & Research Laboratories Ltd., Bangalore; PLG (50:50), PLG (70:30), PLG (80:20) and PLG (90:10) was from Polysciences Inc., PA, USA. Freund's complete adjuvant, Span 60, Tween 80 and triacetin were from Sigma Chemical Co., MO, USA; sesame oil from Indo Biotech Foods Ltd., Bombay; HPLC grade acetonitrile, methanol and methylene chloride from Qualigens Fine Chemicals, Bombay and mefanamic acid from B.P. Noel Chemicals (P) Ltd., Bombay.

Instruments

HPLC system with Rheodyne Loop injector (Model:7125) and a variable wavelength detector (Gilson HM Holochrome) (Gilson Medical Electronics, France) interfaced with a CR-3A integrator (Shimadzu, Japan); 5μ ODS Zorbax Column (4·6 × 250 mm) (Sigma Chemical Co., MO, USA); Guard column packing material pellicular ODS (37–53 μm) (Whatmann Inc., Clifton, NJ, USA); plethysmometer (locally fabricated), optical microscope (Leitz Laborlux D, GmBH, Germany). UV-Vis spectrophotometer (UV-240, Shimadzu, Japan), sonicator (Probe Type VC375, Sonics & Materials Inc., USA).

Experimental animals

Albino Wistar rats obtained from the Central Animal Housing Facility, Department of Pharmacology, Kasturba Medical College, Manipal, India were fed with Rat Feed pellets (Hindustan Lever Ltd., Bombay) and had free access to tap water during the course of the experiments.

Adjuvant-induced arthritis in rats and assessment

The chronic inflammatory condition was induced in one of the hind paws of male albino rats (200–215 g) by an intradermal injection (0·05 mL) of Freund's complete adjuvant into the subplanter surface (Newbould 1963). The oedema produced was regularly monitored and assessed by measuring the volume of the injected paw. The plethysmometer used for this purpose was similar to that of Singh & Ghosh (1968). By this method, a minimal change in paw volume (0·02 mL) could be detected. Upon treatment with diclofenac formulations, anti-inflammatory activity was expressed as percentage inhibition of inflammation (primary response) calculated as follows (Turner 1965):

$$I = 100[1 - (a - x)/(b - y)] \quad (1)$$

where: I is the percent inhibition; y is the mean volume of the right hind paw of control rats before adjuvant injection; b is the mean volume of the same right hind paw on days after adjuvant injection; x is the mean volume of the right hind paw of the treated rats before adjuvant injection and a is the mean paw volume of the same right hand paw on days after adjuvant injection.

Statistics

The primary paw volumes measured were expressed as mean ± s.e. Statistical significance of difference between control and test (treated) groups were calculated by one-factor analysis of variance and multiple comparison were made using the Dunett's *t*-test.

Preparation of PLG (50 : 50) microspheres

PLG (50 : 50), microspheres containing homogeneously dispersed diclofenac were prepared by an oil-in-oil solvent evaporation method (Sturesson et al 1993) with slight modification to suit our laboratory conditions. About 1 g PLG was dissolved in 4·5 mL of acetonitrile (HPLC grade) by stirring. A known quantity of diclofenac was dissolved in 0·5 mL acetonitrile and mixed with the PLG solution. The drug-polymer solution was then added drop-wise to sesame oil (50 g, previously filtered) containing 2% w/w Span 60 in a beaker, under constant stirring (1200 rev min^{-1}). Stirring was continued at 35°C for 2·5–3·0 h until all acetonitrile evaporated. Microspheres formed were collected on Whatmann #1 paper and washed with *n*-hexane three times. Finally the microspheres were washed with distilled water containing 0·1% Tween 80 and vacuum dried. The microspheres were sieved (#120) during the final washing process. By varying the drug : polymer proportion by weight during the preparation, different microsphere batches were formulated (Table 1).

Table 1. Drug : polymer proportion of different microsphere batches.

Formulation No.	Drug : Polymer (by wt.)
DM-1	1 : 2
DM-2	1 : 4
DM-3	1 : 6

Characterization

The size distribution of PLG-diclofenac microspheres was determined by microscopy using a standard objective micrometer (ERMA, Japan). Only microspheres passing through seive #270 (ASTM) were used for further studies.

Drug Content. About 100 mg of the microspheres was dissolved in 5 mL dichloromethane. Then methanol (about 10 mL) was added to completely precipitate the polymer and centrifuged at 4000 rev min^{-1} for 10 minutes. A sample of clear supernatent was suitably diluted with phosphate buffer (pH 7·4) and the diclofenac content was determined spectrophotometrically at 277 nm. The average drug content was expressed per 100 mg of microspheres. Drug entrapment or yield (%) was calculated as follows:

$$\text{Drug yield (\%)} = (\text{Actual drug content in microspheres/Quantity of drug taken initially}) \times 100 \quad (2)$$

Drug release profile in vitro

One hundred milligrams each of DM-1, DM-2 and DM-3 formulations were placed in vials containing 15 mL of 0·01 M phosphate buffer (pH 7·4) and kept on a shaker water bath set at 37°C and 60 oscillations min^{-1}. Clear 0·5-mL samples were withdrawn at predetermined intervals during 4 weeks. The drug released was determined spectrophotometrically at 277 nm against appropriate blank (placebo PLG microspheres treated in the same manner). The experiment was performed in triplicate sets.

Preparation of injectable gel-forming implant systems

PLG (50 : 50) copolymer was used in the formulation which formed a gel matrix immediately on contact with aqueous fluids. Diclofenac was dispersed in such formulations containing triacetin as vehicle. The method employed to prepare these formulations was similar to that adopted by Shah et al (1993) with slight modifications. A solution of PLG 50 : 50 copolymer was prepared by stirring the polymer in triacetin at about 50°C and cooled to room temperature (21°C). A known quantity of diclofenac was incorporated into the PLG solution as a fine dispersion by continuously sonicating (20 kHz, 100 W) for 15 s. By varying the PLG concentration, the following gel-forming systems given in Table 2 were formulated. To study the effect of the drug incorporated as a solution rather than dispersion, an appropriate vehicle for the drug that is miscible with PLG solution was chosen. Hence, the drug was dissolved in propylene glycol which formed about 5·8% (w/w) of the total vehicle employed in 10% w/w gel formulation.

In vitro drug release studies

The formulations containing 50 mg drug were transferred into vials containing 10 mL phosphate buffer (pH 7·4) and placed on a shaker water bath set at 37°C and 60 oscillations min^{-1}. Samples from the aqueous medium above the polymeric gel

Table 2. Formulation of gel-forming systems.

Formulation	Code	Contents	(% w/w)	Triacetin
5% gel	DG-1	5	5	90
10% gel	DG-2	10	5	85
15% gel	DG-3	15	5	80
20% gel	DG-4	20	5	75
10% gel*	DG-5	10	5	80

*Propylene glycol (5% w/w) was included in this vehicle

were withdrawn periodically and analysed for diclofenac content spectrophotometrically at 277 nm. The experiment was carried out in triplicate.

In vivo evaluation

Selected DM formulation were suspended in 0·5% (w/v) carboxymethyl cellulose solution in saline and sterilized by τ-irradiation (1·8 M rad) (Ruiz & Benoit 1991) from ^{60}Co source (Gamatron-R, Siemens, Germany). Its evaluation in vivo was performed on male albino rats bearing adjuvant-induced arthritis. Pharmacodynamic study was performed following single subcutaneous injection of the microsphere suspension (diclofenac = 100 mg kg^{-1}) on the 8th day after adjuvant injection. The percentage inhibition of inflammation was monitored throughout the test period as described earlier. Similarly, a selected gel-forming implantable DG preparation (diclofenac = 100 mg kg^{-1}) was evaluated in vivo pharmacodynamically following single subcutaneous injection of the sterilized the formulation on the 8th day post adjuvant treatment in the rats.

The pharmacokinetic profiles of diclofenac were also studied by determining the plasma concentration of diclofenac simultaneously in the animals treated with DM and DG formulations. Plasma concentration of diclofenac was determined by HPLC (Raja Naresh et al 1995). The estimation procedure involved an internal standard – mefanamic acid, which was extracted into methylene chloride along with diclofenac present in the rat plasma samples. A reverse phase octylsilane (C-18) column with mobile phase acetonitrile:0·1 M acetic acid (70 : 30) and the detector set at 277 nm constituted the chromatographic system. The pharmacokinetic parameters were compared with that of a single dose of plain diclofenac in saline (10 mg kg^{-1}, i.m.) administered to a separate group of arthritic rats.

Results and Discussion

PLG (50 : 50) microspheres

Implantable controlled released drug carriers such as beads and films require surgical procedure or special equipments for implantation. Microbeads, due to their large size (up to 780 μm), cannot be readily administered by injection (Tice et al 1989). In the present work, PLG (50 : 50) biodegradable microspheres containing diclofenac were prepared by *oil-in-oil* solvent evaporation method using different drug : polymer ratios. The microspheres obtained were spherical in shape (Fig. 1). The photomicrograph also illustrates the relatively smooth surfaces of the microspheres. All the microspheres (DM formulations) were prepared under similar stirring conditions (1200 rev min^{-1}).

Table 3 shows the physical characteristics and the diclofenac content of the DM microspheres prepared. It was notable that the size of the microspheres was influenced by the polymer concentration in the dispersion phase during preparation. An increase in the polymer concentration resulted in a larger particle diameter. The higher concentration of polymer may have led to an increased frequency of collisions, resulting in fusion of semi-formed particles. Increasing the concentration of dissolved polymer also increased the viscosity of the organic (acetonitrile), phase, which may have reduced the efficiency of stirring of the solutions. These factors may have contributed to an overall increase in the size of the microparticles. Similar results have been reported by Sturesson et al (1993) and Jeffery et al (1991). As the PLG proportion (by weight) in the dispersion phase of the emulsion was increased (viz., drug : polymer ratio 1 : 2, 1 : 4, 1 : 6), the actual diclofenac content (% w/w) in the resulting microspheres decreased (Table 3). However, an entrappment efficiency of 85% was possible for the DM-2 formulation (drug : polymer ratio 1 : 4 by weight). In spite of increasing the polymer proportion in the DM-3 formulation, the drug entrapment efficiency in the microspheres did not increase, but remained almost similar to that of DM-2, under the same manufacturing conditions.

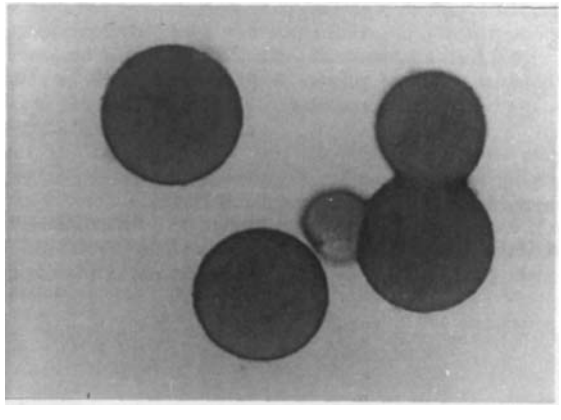

FIG. 1. Photomicrograph of drug-loaded PLG (50 : 50) microspheres (×200).

Table 3. Physical characteristics and diclofenac content of DM microspheres.

Formulation No.	Drug : polymer ratio	Diameter* (μm)	Drug content** (% w/w)	Drug yield (%)
DM-1	1 : 2	71·3 (16·6)	23·05 (0·17)	69·15
DM-2	1 : 4	121·5 (18·9)	17·02 (0·12)	85·10
DM-3	1 : 6	164·7 (24·2)	11·91 (0·39)	83·40

*Mean (± s.d., n = 200); **mean (± s.d., n = 3).

Drug release in vitro

The in vitro release pattern of diclofenac from all the DM formulations showed an initial burst followed by a slow drug release (Fig. 2). The magnitude of initial burst decreased with decreasing drug content in the microspheres. The initial burst may be due to the release of drug located near the microsphere surface. The ensuing phases of slow drug release with DM-1 and DM-2 microspheres were almost linear with respect to time, thus suggesting biphasic drug release profiles. The drug release rate was significantly rapid from DM-1 microspheres which had maximum drug payload (23·05% w/w) and least particle size (55–90 μm). Microspheres with a drug : polymer ratio 1 : 6 by weight (DM-3 formulation) exhibited a triphasic release profile with a secondary burst between the 8th and 10th day. This may be due to collapse of the microspheres (Visscher et al 1988; Sturesson et al 1993), where inside the particles are fused into a rather semi-solid mass as shown in Fig. 3. The in vitro release profiles of DM microspheres, therefore, indicate that the release rates of drug increased as the ratio of drug : polymer increased. These results are in agreement with other published reports (Sampath et al 1992; Iwata & McGinity 1993; Sansdrap & Moes 1993).

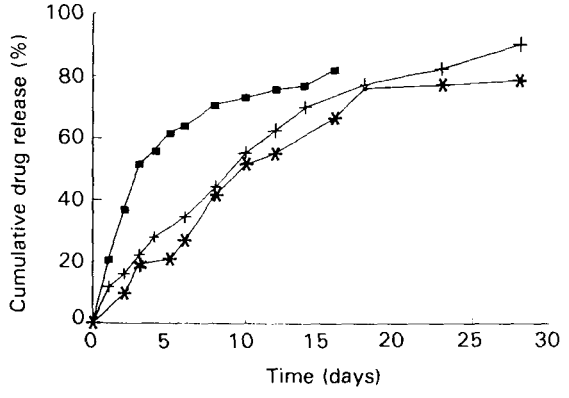

FIG. 2. In vitro release of diclofenac from DM microspheres. Values represent the mean of triplicate results. ■ DM-1, + DM-2, * DM-3. The % drug released from DM-2 was significantly different ($P < 0.05$) from DM-1 during the 2nd to the 12th day of the release period.

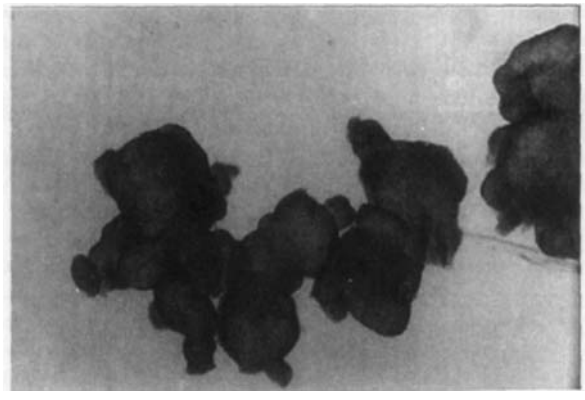

FIG. 3. Photomicrograph showing collapse of drug-loaded PLG (50 : 50) microspheres (DM-3) in vitro (×200).

PLG gel-forming implant system

In the present study, PLG (50 : 50) copolymer has been used in the formulation of in situ forming implant systems for the delivery of diclofenac. This PLG containing formulation was a solution prepared in triacetin (glyceryl triacetate) and the diclofenac was dispersed finely in it so that the formulation could be easily injected through a 22/24 gauge syringe needle. A gel matrix, similar to an implant, was formed immediately on contact with the aqueous environment (0·01 M phosphate buffer, pH 7·4) in vitro. Fig. 4 shows the drug released from DG formulations in vitro.

Formulations DG-1 to DG-4 contained diclofenac dispersed in the PLG solution while DG-5 comprised diclofenac dissolved in propylene glycol which was miscible with the PLG solution. Thus, a study of the physical state in which the drug is incorporated in gel-forming systems was also envisaged. It was observed that the release rates of the dispersed drug from gel matrix of DG formulations were mainly dependent on the PLG (50 : 50) concentration, after an initial burst within 8 hours. It was noted that the burst was more in the case of DG-1, DG-2 and DG-3 formulations with 5, 10 and 15% w/w of PLG (50 : 50) content respectively. On the other hand, the initial burst was substantially reduced when the PLG (50 : 50) concentration was increased to 20% w/w (DG-4) and when the drug was incorporated as a solution (DG-5). Shah et al (1993), have reported similar release characteristics for drugs theophylline and hydrochlorothiazide from PLG gel-forming formulations. They have postulated that when the drug was dispersed rather than dissolved, the release appears to occur not only through partitioning and diffusion through the gel matrix, but also through the porous network of tortuous channels created by the dissolution of dispersed drug. The higher and faster drug release rates observed in the case of DG-1, DG-2 and DG-3 formulations may therefore be due to this dual mechanism of drug release.

In the case of DG-5 formulation, for diclofenac to be released it has to first partition from propylene glycol into the gel matrix from which it could finally get diffused out into the release medium. The drug release rate from DG-5 may hence, be expected to be decreased compared to that from DG-3. It was further noticed that the release profile from DG-5 was

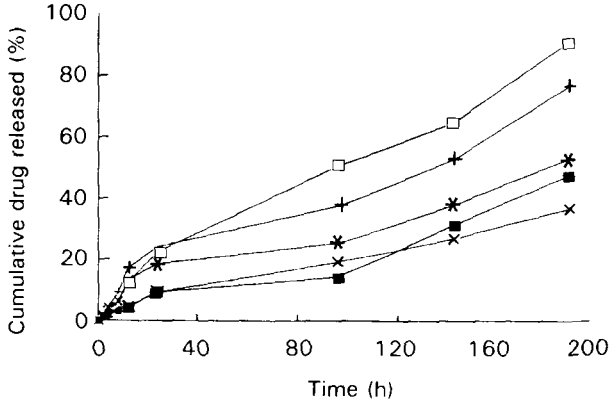

FIG. 4. In vitro release of diclofenac from gel-forming preparations. Values represent the mean of triplicate experiments. □ DG-1, + DG-2, * DG-3, ■ DG-4, × DG-5. The % drug released from DG-3 is significantly less ($P < 0.05$) than that from DG-1 and DG-2 after the 4th day.

almost zero-order after the initial burst, which probably suggests the role of only partition and diffusion mechanisms through the gel matrix. Although DG-1 and DG-2 formulations facilitated the ease of injection due to their lower viscosities, their in vitro release rates were high where about 75% of the incorporated drug was leached out within about 8 days. DG-4 formulation was too viscous due to high (20% w/w) PLG (50 : 50) content and hence, had the disadvantage of less ease of injection. DG-3 formulation offered an optimum ease of injection and the drug release rate also was substantially sustained ($t_{50\%}$ rel = 8 days). Hence the DG-3 formulation was selected for further evaluation in vivo.

Evaluation in vivo

From the in vitro studies, it was found that DM-2 microspheres exhibited optimum characteristics in terms of drug content, yield and drug release profiles. Therefore it was selected for further evaluation in adjuvant-induced arthritic rats. Fig. 5. shows the primary inflammatory response in adjuvant-induced arthritic rats, after a single injection of DM-2 microspheres and DG-3 gel forming matrix (diclofenac = 100 mg kg^{-1}, s.c.). A significant inhibition of inflammation ($P \leq 0.05$) was seen for about 16 and 12 days with DM-2 and DG-3 respectively, from the treatment day. The pharmacokinetic parameters have been shown in Table 4. The plasma t½ (mean terminal half-life) and MRT of diclofenac in the rats was increased to 396 and 557 h respectively, by administering it in the form of DM-2 microspheres (DS = 100 mg kg^{-1}, s.c.), while the values were 14·5 and 33·3 h respectively, for diclofenac administered as a plain solution in saline (10 mg kg^{-1}, i.m.) in rats. It was found that DG-3 provided a higher Cp_{max} (0·552 $\mu g\ mL^{-1}$) than did the DM-2 formulation (0·424 $\mu g\ mL^{-1}$); the respective t_{max} were 96 and 240 h. DM-2 microspheres offered significantly longer ($P \leq 0.05$) duration of effective anti-inflammatory activity (16 days) than DG-3 gel matrix (10 days). Hence, of the selected two injectable implant systems of PLG (50 : 50) copolymer, DM-2 microspheres may be said to be a better formulation in terms of therapeutic activity offered for an extended period of time. However, in situ gel-forming implant systems of PLG (50 : 50) copolymer offer an easy means of preparation with no complex procedures and negligible loss in yield. In addition they can be injected easily. The selection of dosage form can therefore be made based on actual requirements.

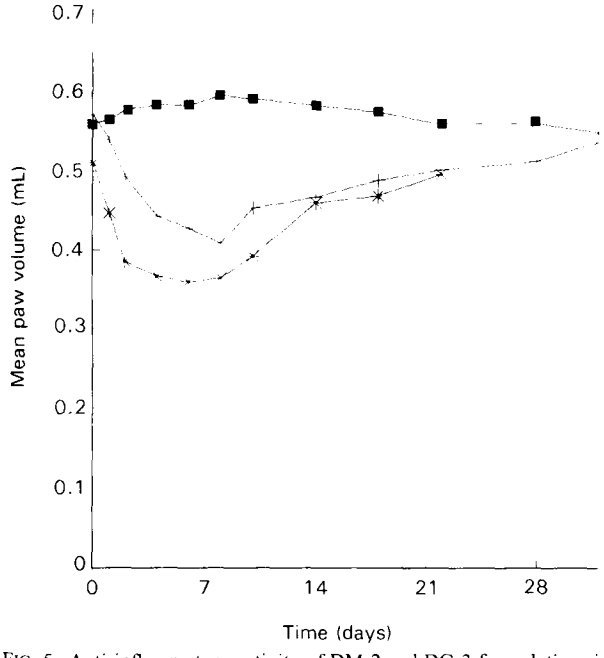

FIG. 5. Anti-inflammatory activity of DM-2 and DG-3 formulations in adjuvant-induced arthritic rats. ■ Control, + DM-2 treated, * DG-3 treated. Values represent the mean of 6 readings in each group.

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Table 4. Pharmacokinetic parameters of diclofenac subsequent to subcutaneous implantation of DM-2 and DG-3 formulations (diclofenac = 100 mg kg^{-1}) in comparison with a single intramuscular injection of plain diclofenac solution (10 mg kg^{-1}) in adjuvant-induced arthritic rats.

Parameters	injection (i.m.)	DM-2	DG-3
Dose (mg kg^{-1})	10·0	100·0	100·0
t_{max} (h)	2·0	240·0	96·0
Cp_{max} ($\mu g\ mL^{-1}$)	2·75	0·424	0·552
K_{el} (h^{-1})	4.79×10^{-3}	1.75×10^{-3}	240×10^{-3}
t½ (h)	14·46	396·1	288·7
$[AUC]_0^{\infty}$ ($\mu g\ h^{-1}\ mL^{-1}$)	51·33	226·12	182·67
MRT (h)	33·33	557·74	296·37

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