J. Pharm. Pharmacol. 1996, 48: 468–473
Received June 28, 1995
Accepted November 21, 1995

Anti-inflammatory Activity of Polyphosphazene-based Naproxen Slow-release Systems

ANITA CONFORTI, SIMONE BERTANI, SABRINA LUSSIGNOLI, LUIGI GRIGOLINI†, MARTA TERZI†, SILVANO LORA‡, PAOLO CALICETI*, FRANCO MARSILIO* AND FRANCESCO M. VERONESE*

*Istituto di Farmacologia and †Istituto di Chimica e Microscopia Clinica, Università di Verona, ‡Istituto di Fotochimica e Radiazioni d'Alta Energia, CNR-Sezionedi Legnaro (Padova), and *Dipartimento di Scienze Farmacologiche, Centro di Studio di Chimica del Farmaco e dei Prodotti Biologicamente Attivi del CNR, Università di Padova*

Abstract

A biocompatible and biodegradable polyphosphazene bearing phenylalanine ethyl ester, imidazole and chlorine (10·7:1:2·5 molar ratio) as substituents of the phosphorus atoms of the polymer backbone was studied for the preparation of polymeric naproxen slow-release systems.

Discs 2·5 cm in diameter and 0·5 mm (thin) or 0·65 mm (thick), loaded, respectively, with 20 and 13·5% naproxen, showed different drug release kinetics, the thin matrices releasing naproxen at a faster rate and for a shorter time. In-vivo studies in rats demonstrated the pharmacological efficacy of these two different delivery systems in the inhibition of acute or chronic inflammatory diseases. Subcutaneous implantation of the thin matrices in rats was found to reduce carrageenan oedema induced both 1 h and 7 days after implantation. Rats implanted with thick matrices showed a reduction in chronic inflammation caused by adjuvant arthritis. Approximately 78% inhibition of arthritic oedema was found 28 days after subcutaneous administration of the matrices whereas 28·7% inhibition was found after daily oral administration of naproxen. Blood levels of naproxen in arthritic rats after matrix implantation showed the presence of drug up to day 28.

These positive results have encouraged us to study a controlled-release system suitable for use in man.

Polymeric drug delivery systems have recently been shown to be useful tools for enhancing the therapeutic performance of several drugs.

Inconvenient pharmacokinetics and low therapeutic index have caused the therapeutic failure of a number of pharmacologically active molecules; this often prevents the widespread exploitation of such drugs. Suitable slow-release dosage forms have therefore been developed in an attempt to find a safer and more rational delivery system for many drugs. Naproxen, for instance, is one of the most widely used non-steroidal anti-inflammatory drugs currently recommended for rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. Despite its fast and quantitative absorption after oral administration, the rapid clearance from the bloodstream significantly affects the dosage schedule, requiring twice-daily administration (Runkel et al 1974). The plasma levels to be expected in man after normal therapeutic doses range from 23 to 49 $\mu g\ mL^{-1}$ (Brogden et al 1975), showing substantial fluctuations. The presence of high minimum and maximum levels of naproxen in plasma could have a negative impact on therapeutic efficacy and on the side effects of the drug, namely platelet aggregation, nephrotoxicity and gastrointestinal and cutaneous effects.

In order to improve the therapeutic potential and safety of naproxen in long-term schedules we are studying the development of slow-release systems for this drug from polymeric matrices. In a preliminary study we investigated the possibility of preparing naproxen slow-release systems using polyphosphazenes as a polymer matrix. The chemical composition of the substituents at the phosphorus atoms has been found to have a substantial effect on the release rate of several drugs, including naproxen (Caliceti et al 1994).

Correspondence: A. Conforti, Istituto di Farmacologia, Policlinico Borgo Roma, 37137 Verona, Italy.

The aim of this study was to evaluate the acute and chronic anti-inflammatory activity of naproxen slow-release systems implanted in rats using two experimental models: carrageenan paw oedema in rats 1 h and 1 week after subcutaneous implantation of thin matrices containing naproxen, and adjuvant arthritis in rats treated with naproxen orally or with implantation of thick matrices. During the development of arthritis, naproxen serum levels were measured at different times by HPLC.

In this study matrices of polyphosphazene substituted with phenylalanine ethyl ester, imidazole and chlorine at the phosphorus atom were prepared in the form of discs. Different thicknesses and drug loadings were used: thin matrices with more entrapped drug were devised for a rapid release in a short time, suitable for an acute model of inflammation such as carrageenan oedema. Thick matrices that release the drug more slowly and for a longer time seem suitable for the treatment of chronic inflammation, e.g. adjuvant arthritis.

The next stage of this study will be the preparation of a system more suitable for controlled-release of non-steroidal antiinflamatory drugs for human use, e.g. microspheres which can be injected by syringe.

Materials and Methods

Chemicals and instruments

Naproxen and carrageenan were purchased from Sigma (St Louis, MO, USA). Heat-killed *Mycobacterium butyricum* was from Difco (Detroit, MI, USA). All the other chemicals and solvents were obtained from Carlo Erba Farmitalia (Milan, Italy). The modular Kontron HPLC system (Milan, Italy) consisted of a model 420 pump, model 460 autosampler, model 430 UV detector (225 nm wavelength) interfaced with a Hewlett-Packard Vectra QS/165 computer (Amsterdam). The

150 × 4·6 mm × 5 μm LC-18-DB column and 20 × 4 mm × 5 μm ODS-Hypersil (C18) guard column were from Supelco (Supelchem, Milan, Italy).

Measurements of paw volumes for anti-inflammatory evaluations were performed using a 7150 water plethysmometer (Ugo Basile, Milan, Italy).

Animals

Male Sprague–Dawley and Lewis rats (Charles River S.p.a., Calco, Italy) with free access to food and water were used for these studies.

Disc matrix preparations

A polyphosphazene bearing phenylalanine ethyl ester, imidazole and chlorine at a molar ratio of 10·7:1:2·5 as backbone phosphorus atom substituents was obtained by a modification of a procedure reported elsewhere (Caliceti et al 1994).

Thin disc matrices (230 mg) containing 0% (control) and 20% naproxen (naproxen:polymer, w/w) were prepared by solvent evaporation (Caliceti et al 1994). The composition of the matrices was: A, 230 mg polyphosphazene and 0 mg naproxen (0% or control); B, 191·6 mg polyphosphazene and 38·4 mg naproxen (20%).

Thick disc matrices (300 mg) loaded with 0% (control) or 13·5% naproxen (naproxen:polymer, w/w) were prepared by solvent evaporation. The composition of the matrices was: A, 300 mg polyphosphazene and 0 mg naproxen (0% or control); B, 264·5 mg polyphosphazene and 35·5 mg naproxen (13·5%).

The in-vitro release of naproxen from thin and thick matrices was evaluated under sink conditions in 10 mM phosphate buffer, 0·15 M NaCl, pH 7·4, as reported previously (Caliceti et al 1994).

Carrageenan-induced paw oedema

Thirty Sprague–Dawley rats weighing 244 ± 15·6 g were used. Ten blank polyphosphazene matrices and ten thin matrices containing naproxen (38·4 mg) were subcutaneously implanted in two groups of ten rats each, through an incision in the flank after anaesthesia with diethyl ether. The control group received no drug. One hour after implantation, paw oedema was induced by injection of 0·15 mL of 1% carrageenan suspended in sterile saline into the plantar surface of the right hind paw. A further carrageenan dose was inoculated into the left hind paw of the same rats 7 days after matrix insertion. The paw volumes were measured 1, 3 and 5 h after each carrageenan injection, and oedema was assessed by the increase in paw volume compared with paw volume measured before injection of irritant. The mean paw swelling in the group of rats treated with matrices was compared with that in the control group.

The response was expressed as percentage inhibition (reduction) of inflammation, using the expression:

$$R = \left(1 - \frac{V_0(V_t' - V_0')}{V_0'(V_t - V_0)}\right) \times 100 \qquad (1)$$

where R is the effect or pharmacological response, V_0 the mean plethysmometric value obtained for the control rats in each set at zero time, V_t the value found for control animals at the predetermined time t and V_0' and V_t' the values obtained under the same conditions for the treated animals (Lauroba et al 1986).

Adjuvant arthritis

Adjuvant arthritis was induced on day 0 in 30 male Lewis rats weighing 192 ± 8·9 g by injection of 0·6 mg of heat-killed *Mycobacterium butyricum,* suspended in 0·1 mL paraffin oil, in the plantar surface of the right hind paw of each rat. The rats were randomly assigned to three groups of 10 animals each. On day 0, ten thick discs without naproxen (controls) and ten thick polymer discs containing naproxen (35·5 mg) were subcutaneously implanted along the flanks of the animals in groups 1 and 2, respectively. The animals in the third group were treated daily with 3 mg kg^{-1} naproxen, suspended in 0·5% carboxymethylcellulose, by oral administration from day 2 until day 18 (12 administrations in total) in order that they should receive the same total amount of naproxen as contained in the thick polyphosphazene films implanted in the animals in group 2. The injected paw volume was measured twice a week up to day 14; that of the contralateral paw was measured from day 10 to day 28.

The effect of naproxen on inflammation was expressed both by paw volume and as percent inhibition of paw oedema observed in the animals treated with naproxen compared to those not treated with the drug, calculated as in carrageenan-induced oedema.

Fourteen, 21 and 28 days after *Mycobacterium butyricum* injection development of arthritis was evaluated by the same observer. Primary and secondary arthritic lesions were scored using an arbitrary scale: left and right hind paw, each 0–7; left and right forepaws, each 0–4·5; tail, 0–5; ears, 0–2; nose and eyes, each 0–1. Animals were weighed twice a week.

In-vivo naproxen HPLC determination

Blood samples were collected by intracardiac puncture 1, 2, 4, 6, 8 and 24 h after the first oral dose in rats treated orally and 3, 24, 96, 120, 192, 288, 360, 456, 528, 624 and 800 h after implantation of naproxen-loaded matrices.

The blood samples were processed as follows: 500 μL of plasma were added to 400 μL 0·025 M phosphate buffer, 0·15 M NaCl, pH 7·4, vigorously stirred for 2 min and centrifuged for 4 min at 2500 rev min^{-1}. After centrifugation, 0·51 mL supernatant was added to 0·34 mL acetonitrile, stirred and further centrifuged for 3 min. The supernatant (20 μL) was then analysed for naproxen content by reversed-phase chromatography using a C18 column isocratically eluted with buffer at pH 7·4 (85% Na_2HPO_4 – 10 mM NaH_2PO_4 – 15% acetonitrile) as the mobile phase. The flow rate was 1 mL min^{-1} and the injection volume 20 μL. The elution profile was detected by UV at 225 nm. A calibration curve was prepared for naproxen; the detection limit was 180 ng mL^{-1}.

Statistical analysis

Student's *t*-test was used for statistical analysis of the data. The non-parametric Mann–Whitney test was used for comparison of the arthritis scores in the different groups.

Results

In-vitro release studies

Fig. 1 shows the kinetics of in-vitro naproxen release from the two kinds of film, differing in thickness and drug loading, used in the study. These matrices were chosen from among several prepared and evaluated in a separate study, using different

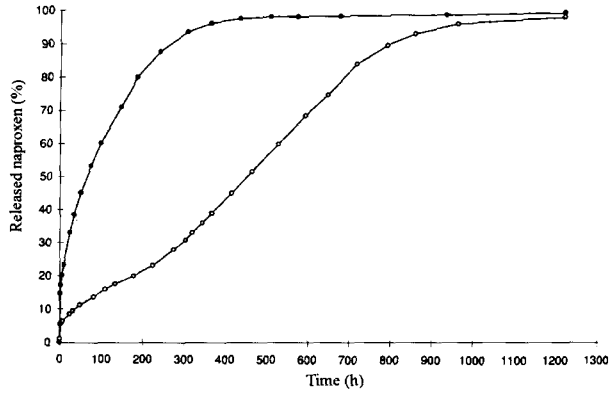

FIG. 1. Cumulative in-vitro naproxen release curve obtained from thin (●) and thick (○) matrices evaluated under sink conditions in 10 mM phosphate buffer, 0·15 M NaCl, pH 7·4. The matrices were prepared by solvent evaporation and had the following composition: thin matrices (●) 191·6 mg of polyphosphazene and 38·4 mg of naproxen (20%); thick matrices (○) 264·5 mg of polyphosphazene and 35·5 mg of naproxen (13·5%). The naproxen released is expressed as percent of the total amount of entrapped drug.

methods of preparation because the kinetics were more suitable for the pharmacological model of implantation employed here.

The figure shows fast in-vitro drug release during the first 200 h from the thin film, whereas the in-vitro release from the thick one is steadier, approaching zero order kinetics and also lasting much longer. The higher release rate obtained with thin matrices is a result of two factors: firstly the lower thickness of the film, and secondly the higher naproxen concentration that leaves large drug crystals on the film surface, as could be seen by scanning electron microscopy (unpublished data).

Carrageenan oedema

Table 1 shows the mean volume of carrageenan paw oedema induced in the same animals 1 h and 7 days after subcutaneous implantation of thin polyphosphazene matrices loaded with naproxen. The data are compared with results obtained in rats subcutaneously implanted with drug-free polyphosphazene matrices to exclude any effect arising from the polymer.

Animals with naproxen-loaded matrices showed a significant reduction in oedema volume 3 and 5 h after injection of carrageenan 1 h after matrix implantation (52 and 29%, respectively), and the inhibition after the 3rd hour also remained significant when carrageenan was re-injected after 7 days (47·8%). The literature data on the anti-inflammatory activity of naproxen in this inflammatory model indicates an ED50 of about 30 mg kg^{-1} (Otterness & Bliven 1985). In our laboratories naproxen (10 mg kg^{-1}) administered orally 1 h before carrageenan caused 32% inhibition of oedema (Cuzzolin et al 1995). The high percentage and persistence of the inhibition achieved by naproxen-loaded matrices confirm the in-vitro studies and are in agreement with the constant level of naproxen in plasma which we found to be maintained by this slow-release system (1·1 $\mu g\ mL^{-1}$ after 10 days).

Adjuvant arthritis

Table 2 gives the mean arthritis scores and the percentage inhibition in rats implanted with thick naproxen-free polyphosphazene matrices (control), thick naproxen-loaded polyphosphazene matrices and rats orally treated with naproxen.

In previous experiments we observed no difference between arthritis scores of animals treated with naproxen-free polyphosphazene matrices and those of untreated rats (data not shown) and therefore considered the group of animals implanted with drug-free polyphosphazene matrices as the control group.

The arthritis scores indicated that the development of arthritic lesions was inhibited by the naproxen-loaded matrices. This inhibition began on day 14 and increased with time, reaching 30·5% inhibition on day 28.

Oral administration of naproxen, on the other hand, proved ineffective at lowering the arthritis score.

For better evaluation of the development of arthritis and the activity of the drug, we measured the oedema arising in the injected paw as the primary reaction to *Mycobacterium butyricum* and the oedema in the contralateral paw as the secondary reaction. Naproxen-containing discs and oral administration of naproxen both reduced the primary response, and to a similar extent; mean inhibition values were 19·3 and 20·5%, respectively, up to day 10 (Table 3).

Fig. 2 shows the volume of the contralateral paw determined at the scheduled times. The effect of the naproxen-loaded matrices increased with time; oedema inhibition was highest on

Table 1. Anti-inflammatory activity of naproxen-loaded matrices in carrageenan-induced oedema. Two groups of rats were subcutaneously implanted with thin naproxen-free matrices (control) or with thin matrices containing 38.4 mg naproxen. The control group received no drug. The mean paw swelling in the group of rats implanted with thin matrices was compared with that obtained in the control group.

		Oedema volume (mL ± s.d.)		
	n	1h	3h	5h
Oedema induced 1 hour after matrix implantation				
Controls	10	0·39 ± 0·11	1·43 ± 0·30	0·96 ± 0·21
Void matrices	9	0·23 ± 0·13	1·11 ± 0·28	0·87 ± 0·16
Naproxen-loaded matrices	10	0·23 ± 0·11	0·53 ± 0·26*** (52·0%)	0·61 ± 0·22* (29·0%)
Oedema induced 7 days after matrix implantation				
Controls	10	0·48 ± 0·15	1·10 ± 0·26	1·28 ± 0·23
Void matrices	9	0·43 ± 0·22	1·42 ± 0·27	1·20 ± 0·25
Naproxen-loaded matrices	10	0·40 ± 0·25	0·74 ± 0·45** (47·8%)	1·10 ± 0·32 (8·3%)

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ compared with the control group (Student's t-test). Percent inhibition in comparison with the control group is given.

Table 2. Arthritis score and inhibition of adjuvant arthritis in rats implanted subcutaneously with thick naproxen-free matrices (control) or with thick matrices containing 35·5 mg of the drug, and in rats receiving oral doses of 3 mg kg^{-1} of naproxen suspended in 0·5% carboxymethylcellulose for a total of 12 administrations in order to receive the same total amount of naproxen as contained in the matrices. The number of rats used is given below each score.

	Day 14	21	28
Control	21·5 (8)	27·0 (8)	27·2 (6)
Naproxen-loaded matrices	17·7* (17·6%) (9)	21·7 (19.6%) (7)	18·9 (30·5%) (5)
Oral naproxen	22·1 (10)	26·0 (3·7%) (10)	26·6 (2·2%) (8)

*$P < 0.05$, significantly different from the arthritic control group (Mann–Whitney U test). Percent inhibition in comparison with the control group is given.

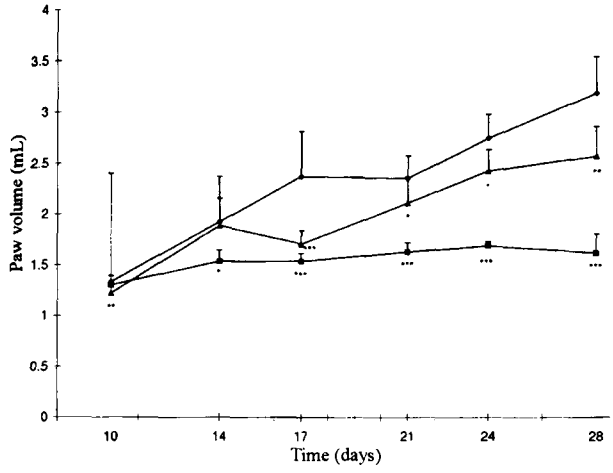

FIG. 2. Contralateral paw volume during adjuvant arthritis in rats implanted subcutaneously with naproxen-free thick matrices (control) or with thick matrices containing 35·5 mg of the drug, and in rats receiving oral doses of 3 mg kg^{-1} of naproxen suspended in 0·5% carboxymethylcellulose for a total of 12 administrations in order to receive the same total amount of naproxen as contained in the matrices. Data are means ± s.d. Control group (◆); naproxen-loaded matrices (■), oral naproxen (▲). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different from arthritic control group (Student's t-test).

day 28 (77·7%, as shown in Table 3). Oral administration of naproxen, on the other hand, showed the opposite trend, with 57% oedema inhibition on day 10 decreased to 28·7% on day 28 (Table 3).

The data obtained following oral administration of naproxen showed greater activity at inhibiting paw oedema volume compared with arthritis score lowering ability, in good agreement with other studies on arthritis previously performed with naproxen in our laboratories (Cuzzolin et al 1995). Other authors found higher activity of oral naproxen in inhibiting adjuvant arthritis, and this might be a result of the use of a different strain of rat and different dosage schedules (Sofia et al 1975; Ackerman et al 1979).

On day 28 all animals were sacrificed; no signs of local irritation were noted at the sites of implantation of the matrices.

Monitoring of naproxen during adjuvant arthritis

The in-vivo release of naproxen from loaded matrices during the development of adjuvant arthritis has been studied by monitoring blood levels of naproxen. The concentration-time curves after oral administration of naproxen and subcutaneous implantation of thick naproxen matrices are reported in Figs 3 and 4, respectively.

Table 3. Inhibition of injected and contralateral hind paw oedema volume during adjuvant arthritis. Two groups of rats were subcutaneously implanted with thick naproxen-free matrices (control group) and thick matrices containing 35·5 mg of naproxen. Another group was treated orally with 3 mg kg^{-1} naproxen from day 2 to 18 (12 administrations in total). The mean paw swelling in the group of rats receiving naproxen was compared with that obtained in the control group. The number of rats is given in parentheses.

Injected paw % inhibition compared with the control group

Days	3	7	10	14
Naproxen-loaded matrices	16·0 (9)	17·3 (9)	24·6 (9)	46·0 (9)
Oral naproxen	16·0 (10)	21·0 (10)	24·6 (10)	25·7 (10)

Contralateral paw % inhibition compared with the control group

Days	10	14	17	21	24	28
Naproxen-loaded matrices	23·8 (9)	51·0 (9)	68·5 (8)	62·2 (7)	67·7 (5)	77·7 (5)
Oral naproxen	57·0 (10)	58.0 (10)	53·2 (10)	30·7 (10)	20·5 (8)	28.7 (8)

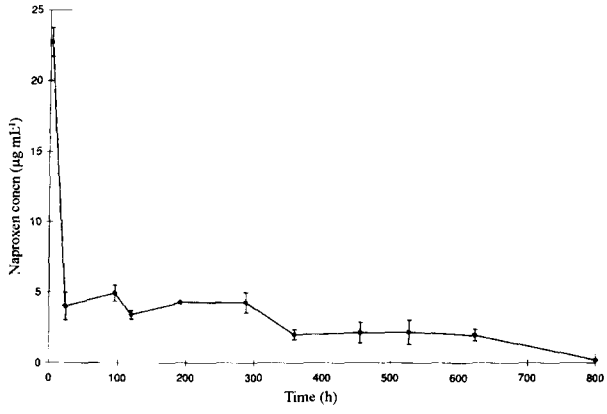

FIG. 3. Blood levels of naproxen released during adjuvant arthritis from thick polyphosphazene matrices, containing 35·5 mg of naproxen, subcutaneously implanted in rats on the same day as induction of arthritis. Naproxen blood levels were determined by HPLC. Each point is the mean result from three animals.

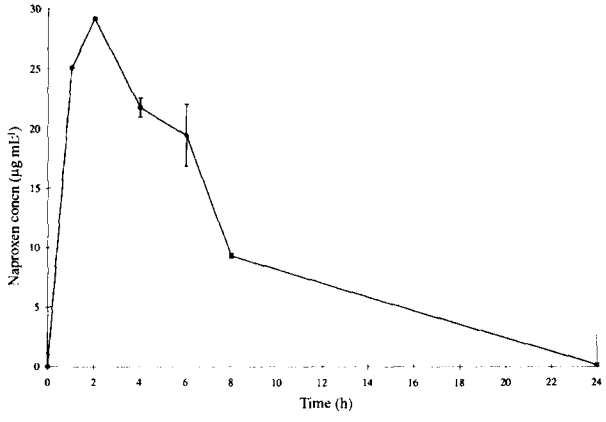

FIG. 4. Blood levels of naproxen determined by HPLC after the first administration of 3 mg g^{-1} naproxen to arthritic rats. Naproxen was administered from day 2 to day 18 (12 administrations in total). Each point is the mean result from three animals.

A single implant of a polyphosphazene matrix containing 35·5 mg of naproxen results in constant blood levels up to day 28. After rapid decrease within the first 24 h of implantation, the levels ranged from 2 to 4·9 $\mu g\ mL^{-1}$. The naproxen concentration – time curve after oral administration in arthritic rats, on the other hand, shows a maximum 29·17 mg mL^{-1} 2 h after administration, the levels rapidly decreasing to zero after 24 h (Fig. 4). The elimination half-life in rats is lower than in humans (6·3 h compared with 12–15 h) and substantial fluctuations in blood levels could be expected during prolonged therapy with once-daily administration.

Discussion

Naproxen is widely used for the treatment of rheumatic and other inflammatory diseases. As the half-life in man is approximately 13 h (Runkel et al 1972), the typical daily dose (from 0·5 to 1 g) is administered in two portions. Once-daily sustained-release naproxen preparations have recently been developed, with several potential benefits (Kelly et al 1989; Pilla et al 1990). Compared with conventional tablets, the controlled-release preparations are convenient for the patient, minimize fluctuations in plasma concentrations and result in a much lower incidence of gastrointestinal side effects.

For the purpose of obtaining release systems capable of guaranteeing therapeutic levels of the drug for several days, polyphosphazene matrices have been studied as naproxen sustained release systems in animals. In this paper the anti-inflammatory activity of matrices was tested in rats during acute and chronic inflammation, namely carrageenan oedema and adjuvant arthritis. The release rates were tailored, by changing the geometry and the drug load of the matrix, to fit the needs of the two inflammation models, one relatively short and the other very long-lasting.

Worthy of note are the biocompatibility and lack of toxicity of polyphosphazenes. This recently enabled us to prepare a tubular prosthesis for channelling nerve regeneration; the prosthesis persisted for several months without toxic effects (Langone et al 1995). Also of importance is the degradation of these polymers to inert products, thus eliminating the need for a second operation to remove the empty matrix (Grollerman et al 1986; Allcock 1990; Caliceti et al 1992).

The results obtained from the experiment on acute inflammation showed significant inhibition of oedema development both 1 h after matrix implantation and one week later. In particular, the persistence of in-vivo activity confirms in-vitro release studies, indicating that thin matrices released about 80% of the drug after 7 days, without causing tachyphylaxis in animals.

In the light of the potential future use of polyphosphazene-based slow-release systems in chronic inflammatory diseases in man, the efficacy of the implantation of more slowly releasing naproxen matrices in inhibiting adjuvant arthritis development up to day 28 is very encouraging. This form of naproxen administration appears to be much more effective than daily oral therapy, considering that at the end of the experiment, the total amount of drug is the same in the two groups of rats. These results are also supported by the blood level–time curves of the drug after oral administration and after matrix implantation. In the first the naproxen blood concentration rapidly decreases, according to pharmacokinetic data in rats indicating plasma half-lives ranging from 4 to 5 h after oral or intravenous administration of 3–5 mg kg^{-1} of naproxen (Runkel et al 1972; Lauroba et al 1986). In animals treated with naproxen-loaded matrices the in-vivo time curve reflects the in-vitro release (80% of drug released after approximately one month) and suggests that relatively low but constant levels of drug are more effective than higher but rapidly decreasing peak levels.

The positive results obtained with naproxen discs in rats encouraged us to study a controlled-release system with the same characteristics as the disc matrices but which are suitable for use in man. This experiment, with microspheres which can be easily administered with a syringe, is at an advanced stage and pharmacokinetic studies have yielded positive findings with the result that therapeutic studies might soon be initiated.

Acknowledgements

This research was partially supported by a MURST (Italian Ministry of the University and Scientific and Technological Research) 40% grant.

References

Ackerman, N. R., Rooks, W. H., Shott, L., Genant, H., Maloney, P., West, E. (1979) Effects of naproxen on connective tissue changes in the adjuvant arthritic rat. Arthritis Rheum. 22: 1365–1374

Allcock, H. R. (1990) Polyphosphazenes as new biomedical and bioactive materials. In: Chasin, M., Langer, R. (eds) Biodegradable Polymers as Drug Delivery Systems. Marcel Dekker, New York, pp 163–193

Brogden, R. N., Pinder, R. M., Sawyer, P. R., Speight, T. M., Avery, G. S. (1975) Naproxen: a review of its pharmacological properties and therapeutic efficacy and use. Drugs 9: 326–363

Caliceti, P., Veronese, F. M., Marsilio, F., Lora, S., Seraglia, R., Traldi, P. (1992) Fast atom bombardment in the structural identification of intermediates in the hydrolytic degradation of polyphosphazenes. Org. Mass Spectrom. 27: 1199–1202

Caliceti, P., Lora, S., Marsilio, F., Guiotto, A., Veronese, F. M. (1994) Amino acid esters and imidazole derivatives of polyphosphazenes: influence on release of drugs, degradability and swelling. Il Farmaco 49: 69–74

Cuzzolin, L., Conforti, A., Adami, A., Lussignoli, S., Menestrina, F., Del Soldato, P., Benoni, G. (1995) Anti-inflammatory potency and gastrointestinal toxicity of a new compound, nitronaproxen. Pharmacol. Res. 31: 61–65

Grollerman, C. W. J., De Visser, A. C., Wolke, J. G. C., Klein, C. P. A. T., Van Der Goot, H., Timmerman, H. (1986) Studies on a bioerodible drug carrier system based on a polyphosphazene. J. Contr. Rel. 4: 133–142

Kelly, J. G., Kinney, C. D., Devane, J. G., Mulligan, S., Colgan, B. V. (1989) Pharmacokinetic properties and clinical efficacy of once-daily sustained-release naproxen. Eur. J. Clin. Pharmacol. 36: 383–388

Langone, F., Lora, S., Veronese, F. M,. Caliceti, P., Parnigotto, P. P., Valenti, F., Palma, G. (1995) Peripheral nerve repair using poly(organo)phosphazene tubular prothesis. Biomaterials 16: 347–353

Lauroba, J., Domenech, J., Moreno, J., Pla-Delfina, J. M. (1986) Relationship between biophasic disposition and pharmacokinetic behavior in nonsteroidal antiinflammatory drugs. Arzneim. Forsch. 36: 710–714

Otterness, I. G., Bliven, M. L. (1985) Laboratory models for testing nonsteroidal antiinflammatory drugs. In: Lombardino, J. C. (eds) Nonsteroidal Antiinflammatory Drugs. John Wiley and Sons, New York, pp 111–249

Pilla, G., Capucci, M., Palazzini, E., Galli, G. (1990) Controlled-release naproxen: an assessment of efficacy and tolerance in arthropathic patients. Curr. Ther. Res. 48: 949–958

Runkel, R., Chaplin, M., Boost, G., Segre, E., Forcelli, E. (1972) Absorption, distribution, metabolism and excretion of naproxen in various laboratory animals and in human subjects. J. Pharm. Sci. 61: 703–708

Runkel, R. A., Forchielli, E., Selevius, H., Chaplin, M., Segre E. (1974) Non linear plasma level response to high doses of naproxen. Clin. Pharmacol. Ther. 15: 261–266

Sofia, R. D., Knobloch, L. C., Vassar, H. B. (1975) Inhibition of the primary lesion of adjuvant-induced polyarthritis in rats (18-hour arthritis test) for specific detection of clinically effective anti-arthritic drugs. J. Pharmacol. Exp. Ther. 193: 918–931