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Implantable Methotrexate films using Poly (E Caprolactone) as Biodegradable Carrier

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Biodegradable films of Methotrexate using Poly (E caprolactone) were prepared. The *in vitro* release was enhanced on incorporation of Poly (glycolic acid). The *in vivo* degradation of the polymer was faster compared to the *in vivo* drug release. The histopathological studies showed no deleterious effects at the site of implantation.

CONTROLLED drug delivery systems have received tremendous attention over the last two to three decades and the significant research interest in the long term maintenance of therapeutic drug levels coincides with the increased medical and public acceptance of such systems.[1,2] Implantable controlled release systems provide advantages over conventional drug therapies[3,4] and the applications of these in developing novel approaches are well reviewed[5,6].

The biodegradability and biocompatibility of polyesters such as poly E caprolactone (PEC), polyglycolide (PGA) and polylactic acid (PLA) and their copolymers have been established from their uses in surgical grafts, implants and various prosthetic devices[7-11] and they do not cause adverse tissue reaction[12-14].

The various properties of the polymeric drug carrier can be used to control the drug release. The permeability of a polymer for a particular drug molecule depends on the polymer composition which can be varied with respect to copolymer ratio, molecular weight, crystallinity.

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PEC is a biodegradable, aliphatic polyester which has been widely used as a biomaterial[15] and in the controlled release of drugs[16,17].

Methotrexate (MTX) is widely used in the treatment of neoplastic disorders. Macromolecular carriers for MTX include microsphere preparation using MTX-albumin conjugate[18], immunoglobulin- MTX conjugate[19], Polylysine-MTX conjugate[20], Depo-MTX using cyclodextrin[21], gelatin-MTX conjugate[22,23]. Other carriers and formulations were niosomes[24,25], polyanhydride microspheres[26] multiple emulsions[27].

The aim of the present investigation is to prepare implantable films of MTX using PEC, to study the effect of addition of PG on the release and the *in vivo* degradation of these polymers. MTX was chosen as the antineoplastic drug because it is cell-cycle phase specific whereby prolonged exposure of the drug to the cancer cells is necessary for optimal efficacy and since these polymers release the drugs for a long period of time they could be effectively utilised for such drugs.

Table 1 : Composition of the films

ABB.	Drug :	Polymer	% PEC	% PG
F16	1	5	—	—
F17	1	4	—	—
F18	1	3	—	—
F19	1	4	90	10
F20	1	4	80	20
F21	1	4	70	30

Table showing the composition of the films prepared by blending drug and polymer in different ratios

MATERIALS AND METHODS

Materials

Poly (E caprolcatone), molecular weight 35-45000 and Poly (glycolic acid) were procured from Polysciences USA. Methotrexate was a gift sample from American Cynamide Co, USA. All other ingredients were of analytical grade.

Animals

For *in vivo* polymer degradation, six to eight weeks old BALB/c mice weighing 20-25 g and for histopathological studies male Albino rats weighing 200-250 g were used from an inbred colony maintained in our animal house under controlled conditions of temperature (23 ± 2°), humidity (50 ± 5%) and light (10 and 14 h of light and dark respectively). The animals were given sterile food prepared in the laboratory as per the standard formulation and filtered water *ad libitum*. Throughout the experiment 5-6 animals were housed in polypropylene cage containing sterile paddy husk as bedding material.

Preparation of films

The films were prepared by the method reported by Medlicott *et al*[28]. PEC was dissolved in dichloromethane with stirring. MTX was dispersed in dichloromethane and sonicated continuously at 100 W, 55000 Hz prior to the addition to the polymer solution. Films were caste onto specially designed rectangular plates lined with aluminium foil. The solvent was allowed to evaporate at room temperature in a vacuum oven. The films were removed and cut into square patches each patch containing (known quantity of drug) 5 mg of the drug.

In order to study the effect of poly glycolic acid (PG) on the release of MTX from PEC-MTX films, a few more films were prepared. PG was included in the PEC-MTX film preparation, at proportion such as 10, 20 and 30% (by weight) of the polymer PEC, keeping drug : polymer ratio (by weight) equal to 20\80 (Table 1).

In vitro release

The films were placed in screw capped vials containing phosphate buffered saline pH 7.4 (PBS). The vials were placed in incubator shaker bath thermostated at 37 ± 1° at a speed setting of 25 cycles per minute. Aliquot was withdrawn at different time intervals, filtered and analysed for the drug content spectrophotometrically at 303 nm.

In Vitro drug release of MTX from PEC films

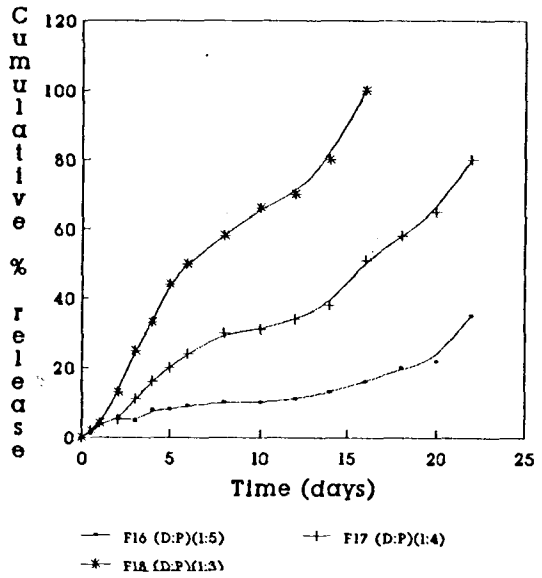

Fig. 1 Graph depicting the *in vitro* release of Methotrexate from films prepared with different ratios of poly E caprolactone

In Vitro drug release of MTX from PEC - PG films

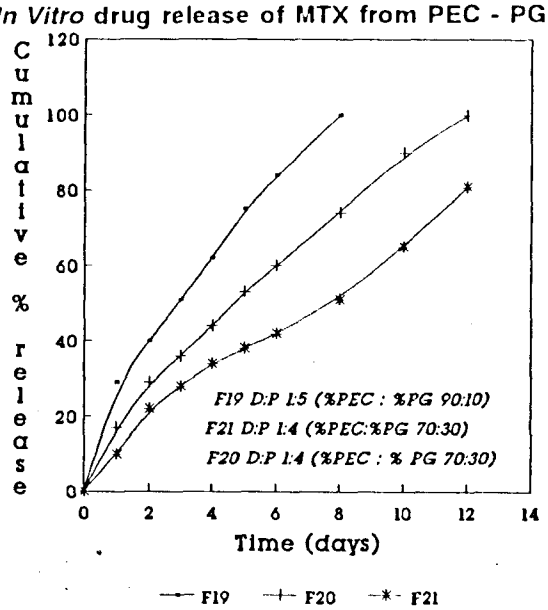

Fig. 2 Graph depicting the in vitro release of Methotrexate from films prepared with different ratios of poiymer blend vis. poly E caprolactone and poly glycolic acid

In vivo polymer degradation and *in vivo* drug release

The degree of *in vivo* polymer degradation was evaluated by measuring the weight loss of the film after subcutaneous implantation of the film in BALB/c mice. At predetermined time intervals the film was surgically excised by killing the mice by cervical dislocation. The films were freed of connective tissues and dried in vacuum.

The degree of *in vivo* degradation was calculated as follows % degraded = [100 (L_o - L) /L_o] where L_o is the weight loss of after degradation, and L is the weight loss of the film after degradation.

The *In vivo* drug release was estimated by excising the film at predetermined time intervals, extracting the drug with PBS pH 7.4 and analysing at 303 nm spectrophotometrically. The difference in the amount of drug prior to the implantation and after release was calculated and cumulative percentage calculated for plotting a graph. Film F20 was used for *in vivo* evaluation.

Histopathological studies

The muscle at the site of implantation was removed and fixed using 10% formalin. The tissues were stained with hematoxylin and eosin and studied for histopathological changes.

RESULTS AND DISCUSSION

In vitro release

Fig. 1 shows the release profile of MTX from PEC films. Films prepared with 25% drug loading broke during the release studies. The films released the drug over a period of 2 to 3 weeks. There was a lag time prior to the burst effect and was maximum for film F18. Medlicott *et al*[28] observed a similar lag time for films prepared with PEC for chlorhexidene diacetate. This lag time can be explained on the basis of the insolubility of the drug in dichloromethne and the formation of a suspension in the organic polymer solution. Since the viscosity is less and the evaporation time of the solvent around 24 to 36 h, it is possible that the suspended particles sedimented

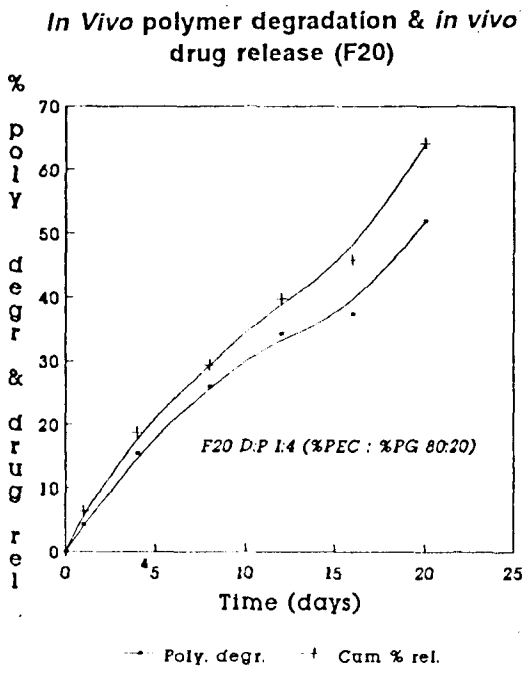

Fig. 3 Graph depicting the *in vivo* polymer degradation and *in vivo* Methotrexate drug release after implantantion of the films in mice

down on the surface of the plates to form a layer of the drug. Since the release was carried out by placing the surface of the film in the same manner as during processing, the molecular diffusional path length may have increased resulting in the lag time. To confirm this finding, the *in vitro* release method was modified according to the method reported by Deasy *et al*[29]. The films were suspended using a nylon filament in vials containing PBS pH 7.4 at 37°C ± 1°C and agitated at 25 cycles per minute in horizontal shaker bath. It was observed that the lag time was no longer evident.

The release data was fitted in first order kinetics and square root time equation (Higuchi[30]) and it was found that the r^2 values were higher (> 0.93 except for F19 r^2 0.8) for square root time equation than first order (r^2 0.68 to 0.95). The r^2 were not very high indicating that although the drug release is by diffusion controlled mechanism some other mechanism may also contribute to the drug release. The release of the drug was not irregular which is in contrast to Pitt *et al*[31] and factors likes drug solubility, processing conditions, thickness, surface area etc. may contribute to this observed difference.

The permeability of PEC films were sufficiently low and hence have been suggested for developing sustained drug delivery systems[31]. Studies on the drug release rates from films of co-polymers of E Caprolactone with glycolic and DL-Lactic acid were carried out by Pitt *et al*[32]. It was found that films of E Caprolactone - glycolic acid (90:10) and E Caprolactone - DL - Lactic acid (50:50) co-polymers did not provide significant different drug release compared to pure PEC films. However, the drug release rates from PEC films could be manipulated by using PEC in physical mixtures with another polymer like poly vinylacetate[16,33,34]. Therefore a preliminary study of the influence of PG on the release profile of MTX from PEC films was investigated. The release profile is shown in Fig. 2. The drug release rate was substantially influenced by the proportion of PG. The release rate increased and was proportional to the weight % of PG in the film composition. The enhanced release in the presence of PG may be due to the more hydrophylic component PG and this facilitates the penetration of the medium into the matrix whereby increase in drug dissolution\diffusion rates[35]. The drug release rates were rapid ($t_{50\%}$ = 3, 5, 7.8 days for F19, F20, F21 respectively) and these films do not justify significantly the purpose of implantable sustained release drug delivery systems.

In vivo degradation and drug release

The polymer degradation and the release are shown in Fig. 3. The release of the drug was faster than the polymer degradation were. The time required for 50% drug release and polymer degradation was 16.5 and 19 days respectively. Imasaka et al[36] have reported the *in vivo* degradation and the release for hydrophylic and hydrophobic drugs incorporated in PEC and Poly (δ valerolactone) and have found that for hydrophobic drugs the release and the deg-

radation were almost same. The slow degradation may be due to the poor ability of the hydrophobic drugs to form porous structure in the polymer because they are not easily dissolved, leading to slow hydrolytic cleavage of the ester linkage, autocatalysed by the carboxylic end groups of the polymer[37-39].

Heya et al[40] have reported that the drug release from Poly (lactic-co-glycolic acid) loaded thyrotropin releasing hormone microspheres was faster than the polymer degradation suggesting that the drug is able to diffuse through pores and channels in the polymer matrix especially during the advanced stages of biodegradation. Our results were similar and can be explained on these basis.

Histopathological studies

A transient acute inflammatory response was observed which had terminated by the end of 3 days. This was basically due to the surgical implantation. Similar inflammatory response was observed by Woodward et al[15] which had terminated by the 6th day. The histopathological studies of the muscle tissue around the site showed no change in shape, size or arrangement of muscle fibres or connective tissues.

REFERENCES

1. Langer R., **Science**, 1981, 240, 1527.
2. Tice, T.R., **Polymer Preprints.**, 1985, 26, 198.
3. Danckwerts, M., and Fassihi, A. **Drug. Dev. Ind. Pharm.**, 1991, 17, 1465.
4. Chien, Y.W., In **Novel Drug Delivery Systems.**, Marcel Dekker, New York, 1982, 311.
5. Wise, D.L., Gregory, J.B., Neuberne, P.M., Barthlow, L.C., and Stanbury, J. In Kostelnik, R.J. Ed. **Polymeric Delivery Systems.**, 1978, 121.
6. Leong, K.W., and Langer. R. **Adv. Drug. Del. Rev.**, 1987, 1, 199.
7. Chu, C.C. **J. Biomed. Mater. Res.**, 1982, 16, 117.
8. Fredericks, R.J., Melveger, A.J., and Dolegiewitz. **J. Polym. Sci. Polym. Phys. Ed.**, 1984, 22, 57.
9. Zimmerman, M., Parsons, J. R., and Alexander. H. **J. Biomed. Mater. Res. Appl. Biomater. Suppl.**, 1987, 21, 345.
10. Chegini, N. Hay, D.L., Von Fraunhofer, J.A., and Masterson., B.J. **J. Biomed. Mater. Res.**, 1988, 22, 71.
11. Hay, D.L., Von Fraunhofer, J.A., Chegini, N., and Masterson., B.J. **J. Biomed. Mater. Res.**, 1988, 22, 179.
12. Thies, C. **Int. J. Pharm. Tech. Prod. Mfr.**, 1981, 2, 25.
13. Leung, S.S., Lee, V.H.L., and Robinson, J.R. In Robinson, J.R. Lee VHL Eds., **Controlled Drug Delivery.**, 1987, 433.
14. Gourlay, S.J., Rice, M.R., Hegyeli, A.F., Wade, W.R., Dillon, J.G., Jaffe, H., and Kulkarni, R.K. **J. Biomed. Mater. Res.**, 12, 1978, 12, 219.
15. Woodward, S.C., Brewer, P.S., Moatamed, F., Schindler. A., and Pitt, C.G.J. **Biomed Mater. Res.**, 1985, 19, 437.
16. Wang, P.J., **Biomed. Mater. Res.**, 1989, 23, 91.
17. Yolles, S., Leafe, T.D., and Meyer, F.J. **J. Pharm. Sci.**, 1975, 64, 115.
18. Kim, C.K., and Oh, Y.K. **Int. J. Pharm.**, 1988, 47, 163.
19. Ghosh, M.K., Dane, K.O., and Mitra, A.K. **Drug Design. Del.** 1988, 4, 13.
20. Shen, W.C., and Ryser, H.J.P. **Mol. Pharmacol.**, 1979, 16, 614.
21. Chatelut, E., Suh, P., and Kim, S. **J. Pharm. Sci.**, 1994, 83, 429.
22. Narayani R., and Rao, K.P. **Int. J. Pharm.**, 1993, 95, 85.
23. Narayani R., and Rao, K.P., **Int. J. Pharm.**, 1996, 128, 261.

24. Azmin, M.N., Florence, A.T., Handjani Vila, R.M., Stuart, J.F.B., Valnerberghe.,and Whittaker, J.S., **J. Pharm. Pharmacol.**, 1985, 37, 237.

25. Chandraprakash, K.S., Udupa, N., Uma Devi, P., and Pillai, G.K., **Drug Delivery.**, 1993, 1, 133.

26. Bhagat, H.R., Hollenbeck, R.G., Pande, P.G., Bogdansky, S., Fait, C.D., and Rock, M. **Drug. Dev. Ind. Pharm.**, 1994, 20, 725.

27. Law, S.L., and Lin, F.M. **Drug. Dev. Ind. Pharm.**, 1991, 17, 919.

28. Medlicott, N. J., Jones, D.S., Tuckeer, I.G., and Holborow, D. **Int. J. Pharm.**, 1992, 84, 85.

29. Deasy, P.B., Finan, M.P., Klatt, P.R., and Hornykiewytsch, T. **Int. J. Pharm.** 1993, 89, 251.

30. Higuchi, T. **J. Pharm. Sci.** 1963, 52, 1145.

31. Pitt, C.G., Jeffcoat, A.R., Zweidinger, R.A., and Schindler, **J. Biomed. Mater. Res.**, 1979a, 68, 497.

32. Pitt, C.G., Gratzl M M Jeffcoat, A.R., Zweidinger, R.A. and Schindler. **J. Pharm. Sci.**, 1979b, 68, 1534.

33. Huatan H., Collett, J.H., Attwood, D., and Booth C. **J. Pharm. Pharmacol.**, 1994, 46, 1038.

34. Creque, H.M.,Langer, R., and Folkman, J. **Diabetes.**, 1980, 29, 37.

35. Gilding, D.K, and Reed, A.M. **Polymer**, 1979, 20, 1459.

36. Imasaka K., Yoshida, M., Fukuzaki H., Asano M., Kumakura, M., Mashimo. T., Yamanaka, H., and Nagai, T. **Chem. Pharm. Bull.**, 1991, 39, 2096.

37. Schindler, A., Jeffcoat, R., Kimmel, G.L., Pitt, C.G., Wall, M.E.,and Zweidinger, R. **Proceeding in 8th Bienneal Polymer Symposium, American Chemical Society,** 1977.

38. Pitt, C.G., Chasalow,F.I., Hibionada, Y.M., Klimas, D., and Schindler, A., **J. Appl. Polym. Sci.**, 1981, 26, 3779.

39. Pitt, C.G., Gratzl, M.M., Kimmel, G.L., Surles, J., and Schindler, A. **Biomaterials.**, 1981, 2, 215.

40. Heya, T., Okada, H., Ogawa, Y., and Toguchi, H. **Int. J. Pharm.** 1991, 72, 199.