RESEARCH PAPER

Received 3rd April, 1997
Accepted 26th July, 1997
Indian J. Pharm. Sci., 1997, 59, (5)pp. 246-250

Implantable drug delivery systems for Centchroman

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Subdermal implantable films of Centchroman were prepared using Poly-E-Caprolactone and Cellulose polymers. They were evaluated for physico-chemical characteristics, *in vitro* drug release profiles in phosphate buffered saline (pH 7.4), stability on storage at room temperature, 37° and 82.5% relative humidity and light exposure. *In vivo* anti-fertility activity of Poly-E-Caprolactone film was evaluated in female albino rats by implanting the film in the flank region. It was evident from this study, that, Poly-E-Caprolactone film was more promising for the development of a bioerodable, long acting contraceptive formulation.

CENTCHROMAN is a non-steroidal, orally effective, post-coital anti-fertility agent which is also in phase III clinical trials for the treatment of advanced breast cancer.[1,2] The present study was aimed at preparation of long-acting, implantable films of Centchroman and testing for activity. Poly-E-Caprolactone (PCL: MW = 25,000 to 35,000) and Hydroxy Propyl Methyl Cellulose (HPMC)/Ethyl Cellulose (EC) were used as the matrices for drug incorporation. PCL is a biocompatible/bioerodable poly(hydroxy acid) polymer, the subdermal film of which would be bio-absorbed from the site of implantation by the time drug depletion is complete.[3,4] HPMC/EC are bio-compatible/non bio-erodable Cellulose polymers which can accommodate a large amount of drug and provide a controlled drug release over a period of time[5]. Carbopol was also used to alter the release profiles.

MATERIALS AND METHODS

Centchroman was received as a gift sample from Torrent Pharmaceuticals Limited, Ahmedabad. PCL was obtained from Polysciences, Inc., Warrington, PA. HPMC (15 cps) was obtained from Shin-Etsu Chemicals Co. Ltd., Japan. EC was obtained from Robert Johnson, Mumbai. Carbopol (974P NF) was obtained from The BF Goodrich Co., Cleveland, OH. All reagents were of analytical grade.

Preparation of Films[6] : PCL films (Drug: Polymer = 1:15) were prepared by dissolving the drug and the polymer in dichloromethane (DCM) and then pouring into specially designed glass moulds (5x8 cm) and allowing to dry at room temperature. Films were cut in strips of 0.5 x 0.5 cm, each strip containing about 1mg of drug (PCL I). For the films of PCL with Carbopol, 30mg of Carbopol was dispersed with the drug and the polymer solution in DCM with the aid of sonication (PCL II).

HPMC/EC (1:1, 1:2, 2:1, HPMC I, HPMC II, HPMC III respectively) films were prepared by the same method using ethanol as solvent. HPMC/EC (1:2) films were also prepared with Carbopol (60 mg) (HPMC IV).

Physico-chemical properties: Film thickness, weight variation, drug content uniformity and tensile

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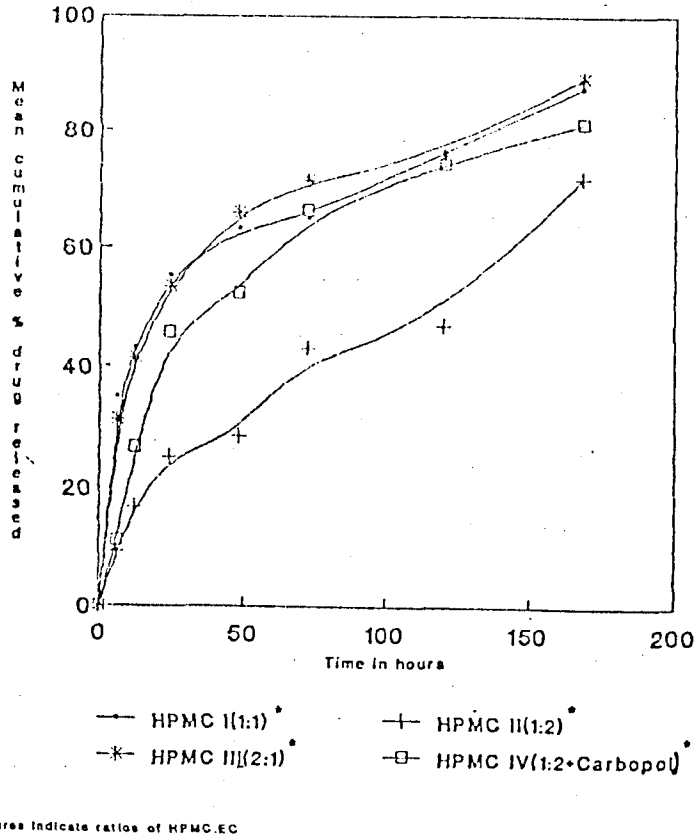
Table 1 : Physicochemical characterization of films of Centchroman

Film	Thickness "mm"	Weight of each strip (0.5 x 0.5 cm) "mg"	Drug content "mg/strip"	Tensile strength "kg/cm^2"
HPMC I	0.835 ± 0.04	15.3 ±0.1	0.910 ± 0.03	654.64
HPMC II	0.828 ± 0.05	14.8±0.2	0.920±0.04	662.23
HPMC III	0.832±0.04	15.2±0.1	9.919±0.04	649.03
HPMC IV	0.841±0.03	16.4±0.1	0.897±0.06	639.38
PCL I	0.710±0.05	15.7±0.2	0.908±0.04	-
PCL II	0.721±0.03	16.1±0.3	0.911±0.02	-

Results are average of 5 films



*Figures indicate ratios of HPMC:EC

Fig. 1: *In Vitro* drug release from HPMC/EC films

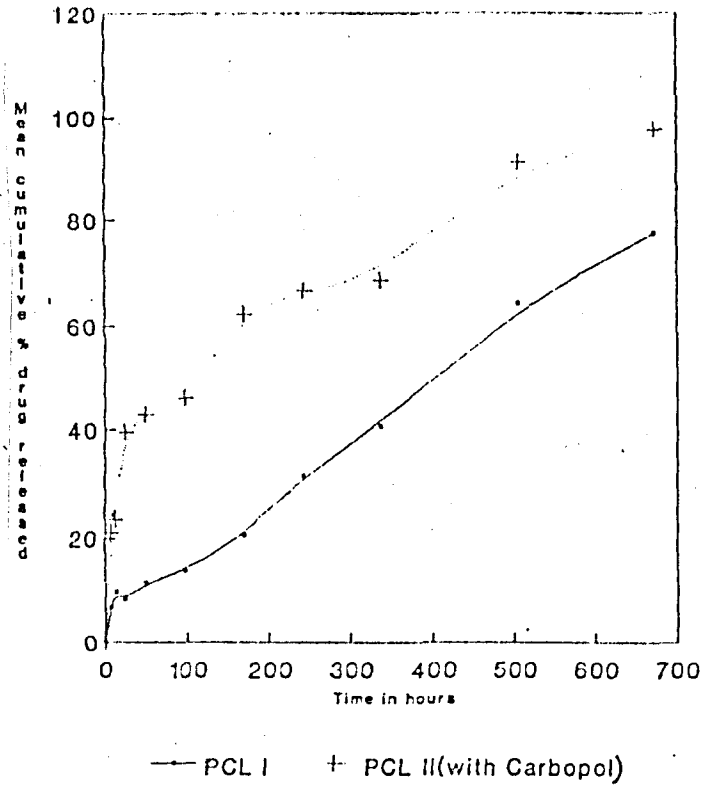


Fig. 2: *In Vitro* drug release from PCL films

strength were found out for 5 films individually and average was taken[7].

***In vitro* release studies:** Each strip containing approximately 1 mg of the drug was kept in a vial containing 10 ml of phosphate buffered saline (PBS pH 7.4). Samples (2 ml) were withdrawn periodically

Table II : Stability studies : Degradation rate constant (K) values of Centchroman in different formulations at different storage conditions

Formulation	Degradation rate constant (K) in day^{-1}			
	Room temp.	37°C	82.5% R.H.	Light exposure
Centchroman	$8.44x10^{-4}$	$4.06x10^{-3}$	$1.05x10^{-2}$	$2.22x10^{-2}$
HPMC I	$1.64x10^{-3}$	$6.31x10^{-3}$	$1.05x10^{-2}$	$1.98x10^{-2}$
HPMC II	$8.44x10^{-4}$	$3.53x10^{-3}$	$9.97x10^{-3}$	$1.74x10^{-2}$
HPMC III	$6.91x10^{-4}$	$3.37x10^{-3}$	$9.13x10^{-3}$	$1.58x10^{-2}$
HPMC IV	$8.44x10^{-4}$	$3.14x10^{-3}$	$8.29x10^{-3}$	$1.52x10^{-2}$
PCL I	$6.91x10^{-4}$	$2.22x10^{-3}$	$3.53x10^{-3}$	$1.32x10^{-2}$
PCL II	$6.14x10^{-4}$	$2.22x10^{-3}$	$3.53x10^{-3}$	$1.32x10^{-2}$

Table III : Anti-implantation effect of Centchroman in different formulations

Formulation	Dose/ route	No. of implantation site of 20th day of pregnancy		No. of rats with no implant-ation sites	% of anti-fertility activity
		No. of rats showing implantation	No. of implantation site in individual rats		
Control (PBS)	0.5 ml i.m.	6	8,7,10,9,12 8	-	0%
Drug dispersed in PBS	5 mg/ kg i.m.	2	2,6	4	66.66%*
PCL-Carbopol film	1mg s.c.	-	-	6	100% **

No. of animals/group = 6

By Chi-square test --

* Significance level P values < 0.05

** Significance level P values < 0.01

with replacement, mixed thoroughly with 3 ml of methanol and analyzed spectrophotometrically at 276 nm). The percentage drug released was found out by extrapolation from the calibration curve of the drug in the same medium (60% methanol: PBS).

Stability studies[8]: Pure drug as well as the films containing the drug were stored at room temperature, 37°, 82.5% relative humidity (R.H.) and exposed to sunlight for a period of one month and evaluated for physical changes, drug content and *in vitro* drug release pattern. The degradation rate constants at different conditions of storage were calculated. The results were analyzed statistically using Graph PAD software (version 1.13)

***In Vivo* Anti-fertility studies:** The method of Khanna and Chaudhury[9] was employed to evaluate the anti-implantation activity of films containing Centchroman. Adult female albino rats, each weighing between 125 - 150 g, showing regular 4-5 days oestrous cycle were divided into 5 groups each containing six rats. They were housed in separate cages, each cage containing three rats. All the animals were fed with a commercial laboratory diet and water *ad libitum* throughout the study. One group of female rats were given PBS as intra-muscular injection into the gluteal muscle; the second group received Centchroman dispersed in PBS and the third group received Centchroman in PCL-Carbopol film as subdermal implant (implantation was done by surgical insertion of the film in the flank region). Young male rats (body weight of each being 200-225 g) of proven fertility were admitted to each of the cages so that the ratio between females to males is 3:1. The males were kept overnight and were seperated each morning. Vaginal smears were taken daily between 10-00 a.m. and 12-00 noon. The day on which thick clumps of spermatozoa were detected in the vaginal smear, was termed as day 1 of pregnancy. The Pregnants were isolated, and hosted in separate cages for gestation. The pregnant female rats were autopsied on 20th day of pregnancy and checked for number of implantations. Histological sections of the ovary and uterus (isolated after autopsy) were taken, stained with Hematoxyl in/Eosin and observed for changes. The muscle tissue at the site of implantation was taken, stained with Hematoxylin/Eosin and examined for histopathological changes.

RESULTS AND DISCUSSION

The physicochemical characters of the films (Table I) showed that HPMC films had good tensile strength, thickness and flexibility compared to PCL films. But they did not have smooth surface and were not uniform in thickness. Conversely, PCL films were very brittle (hence tensile strength could not be determined), non- porous and smooth.

***In vitro* release studies (Figs. I and II):** HPMC films showed a faster release rate than PCL films. Among HPMC films, all showed an initial burst effect followed by a steady release. Incorporation of Carbopol in HPMC films did not improve the release profile (of HPMC IV compared to others) (Fig. I). All the films dissolved slowly in the medium. PCL films on the other hand exhibited a sustained release over 3 weeks. Films with Carbopol (PCL II) showed an initial burst unlike one without Carbopol. However, the release pattern was more steady with PCL film without Carbopol (Fig. II).

Stability Studies: Centchroman drug at room temperature, 37° and 82.5% R.H. for one month revealed no significant degradation ($P \leq 0.05$). Upon light exposure, the degradation rate was slightly accelerated (about 50% decomposition in 30 days). All the films however, were successful in lowering the degradation of the drug (Table II) as evident from the degradation rate constants. This protective effect was more prominent for PCL films under all storage condition.

In vitro release profiles of the films were not altered significantly ($P \leq 0.05$) specially upon storage at room temperature, 37° and 82.5% R.H. (except for HPMC films). On exposure to sunlight, the rate of release was increased. The alteration of *in vitro* release rate was minimum for PCL films at all storage conditions.

***In vivo* studies:** The *in vivo* anti-fertility studies showed that the PCL-Carbopol films of Centchroman were efficient in preventing implantation

(100% protection) as compared to the drug given as PBS dispersion (66.66% protection). The anti- implantation activity was highly significant ($p \leq 0.05$) (for PCL-Carbopol film) and significant ($p \leq 0.05$) (for drug dispersed in PBS) as per Chi-square test (Table III). This was also supported by the histopathological studies, as the sections of treated uterus showed *edematous and degenerated endometrium*, a condition unfavourable for implantation. However, the ovarian structures of the treated groups remained normal. It was also noticed that, none of the animals treated with the formulation exhibited abnormal behavior/deleterious effect externally (like edema/inflammation at the site of implantation). The PCL film was totally biodegraded within 20 days and the skin was normal. The histopathological study of the skeletal muscle tissue around the film implanted site showed no deleterious effect (like changes in shape, size or arrangement of muscle fibres of connective tissue).

In conclusion, the *in vitro* and *in vivo* studies in rats indicate that PCL film is a potential, prolonged release, implantable, bio-degradable contraceptive drug delivery system for Centchroman.

ACKNOWLEDGEMENT

The authors are thankful to Torrent Pharmaceuticals Ltd. for the gift sample of centchroman.

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