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Sustained Release Implants of Chloroquine Phosphate for Chemoprophylaxis of Malaria

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Implants of chloroquine phosphate (CQP) using the biocompatible polymer ethylcellulose (EC) along with or without the addition of excipients l ke PEG 6000, stearic acid and Tween 80 were prepared.These implants were evaluated to assess their physicochemical properties and *in vitro* release profiles. Of the various implants studied, CQP loaded EC implants containing stearic acid were found to provide the maximum retardant effect and hence were selected for further investigations.These implants were then sterilized using Cobalt 60 gamma radiation. Antimalarial efficacy of these implants against *Plasmodium berghei*-infected mice was also investigated.

Inspite of the several insecticides and antimalarial drugs available, malaria is still a major health problem worldwide, especially in the third world countries. Because of the increased spread of chloroquine-resistant *P. falciparum* and toxicity of alternative drugs, it has become extremely difficult to propose simple, widely applicable and uniformly acceptable recommendations for malaria prophylaxis. However, inspite of the shortcomings of the present day antimalarials, prophylaxis may provide the travellers with some degree of protection and it is recommended that travellers to endemic areas should carry an appropriate chemoprophylactic regimen for malaria[1,2]. World Health Organization (WHO) recommends that for antimalarial prophylaxis the drug formulations should be an injectable and provide protection for at least 3 months[3]. Various efforts to prolong the duration of action of antimalarial drugs include preparation of pamoate salts of cycloguanil and pyrimethamine, prodrugs of dapsone and sulphadimethoxine, nonbiodegradable silicone rubber implants of various antimalarials that include primaquine, chloroquine, cycloguanil, sulfadiazine, mefloquine and biodegradable dihydropyran implants containing 20% pyrimethamine and 20% sulphadiazine that were found to protect mice against *P. berghei* for 20 and 35 weeks respectively[4-6].

CQP was chosen as the drug for the present study because it is the most widely prescribed antimalarial drug in the world. It is inexpensive, widely available, well tolerated and cures malaria with relatively few doses. Despite relentless advance of drug resistance in *P. falciparum,* CQP remains the drug of choice for malaria[7,8]. EC is a biocompatible polymer and its use has been reported in parenteral delivery of drugs such as cisplatin and nonreleagnine hydrochloride[9,10].

MATERIALS AND METHODS

CQP was obtained from IPCA, Labs Pvt Ltd., Mumbai, EC (20 cps) was purchased from Colorcon Asia Pvt. Ltd., Mumbai, Tween 80, and Stearic acid were purchased from Loba Chemie, Mumbai PEG 6000 was purchased from HICO Products Pvt. Ltd. Mumbai. All other reagents and solvents used were of analar grade.

Preparation of implants[11]:

Implants of CQP (sieved through 300 mesh) in different ratios, with or without the addition of excipients like PEG 6000, stearic acid and Tween 80 were prepared as presented in Table 1. EC (5 g for 1:1 ratio and 7.5 g for 1:1.5 ratio) was dissolved in 50 ml toluene with the help of overhead propeller stirrer (Remi)

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Table 1 : FORMULATION OF IMPLANTS

Batch	Drug : Polymer ratio	Polymer concentration	Excipients
A	1:1	10% w/v	40% w/w PEG 6000 as plasticizer and 4 drops of Tween 80 as distributing agent
B	1:1	15% w/v	—
C	1:1.5	15% w/v	—
D	1:1.5	15% w/v	40% w/w PEG 6000 as plasticizer and 4 drops of Tween 80 as distributing agent
E	1:1.5	15% w/v	10% stearic acid as release retardant and 4 drops of Tween 80 as distributing agent
F	1:1.5	15% w/v	20% w/w stearic acid as release retardant and 4 drops of Tween 80 as distributing agent

Implants of CQP using the polymer EC, were prepared by solvent casting, using toluene. Varying drug : polymer ratios were tried ; PEG 6000 - plasticizer, Tween 80 - distributing agent, Stearic acid - release retardant.

operating at a moderate speed. The drug powder (5 g for 1:1 ratio and 5 g for 1:1.5 ratio) was dispersed slowly into the above solution and stirred for 5 min. 4 drops of Tween 80 were added wherever required and 2 ml of this dispersion was then added dropwise with the aid of 5 ml pipette into circular teflon moulds (2.5 cms in diameter) placed on a release liner fixed on to a glass plate kept on a perfectly horizontal surface. The moulds were covered with sieves and left to air dry overnight, for evaporation of the solvent. The drug loaded polymer films were obtained in the form of circular discs. The excipients such as PEG 6000 and stearic acid if used were dissolved in warm toluene prior to addition of EC. The edges of the discs obtained on drying were cut and smaller discs of about 5.45 mm diameter were cut by means of a paper punch. These small discs were used as implants. Blank implants were prepared in similar manner without the drug. Selected implants were evaluated further to assess their physicochemical properties.

Physicochemical evaluation of implants:

Colour, odour and visual appearance of the implants were noted. Uniformity of diameter of the implants was assessed using vernier callipers (least count-0.05 mm) and uniformity of thickness using a screw gauge (least count-0.005 mm). Weight variation was assessed using Stanton 461AL electronic balance.

The drug content was determined by measuring the absorbances of suitably diluted water extracts of the implants. The individual implants were weighed and placed in separating funnels to which 20 ml ethyl acetate was added. To this 75 ml distilled water was added and the separating funnels were shaken vigorously. The two phases were allowed to separate overnight. The lower aqueous phase was collected, filtered through Whatman

filter paper No. 1. The absorbances of the diluted extracts were determined at 343 nm, against suitably prepared blanks. The drug concentration was assessed by extrapolation on the standard plot of the drug in distilled water.

In vitro release studies:

The *in vitro* release of CQP from the implants was determined over a period of 7 days in pH 7.4 buffered saline by static method. The individual implants were weighed and immersed in 10 ml of pH 7.4 buffered saline (prewarmed to 37°±2°) in glass vials fitted with rubber stoppers. These vials were maintained at 37°±2° during the entire period of the study. At periodic intervals of time (1,2,4,6, and 24 h, on the first day) every 24 h, for 11 days i.e. 264 h, the buffer from each vial was withdrawn and replaced with fresh buffer. The absorbances of the sampled solutions were measured at 343 nm against suitably prepared blanks. The drug concentration of the solutions were assessed by extrapolation on the standard plot of the drug in pH 7.4 buffered saline. Release studies on uncoated CQP were also carried in the same manner for comparison. From the results of *in vitro* studies, only implants of batch E were selected for sterilization and *in vivo* studies.

Sterilization studies:

Batch E implants, suitably packed in resealable polythene bags were sterilized using Cobalt 60 gamma radiation (Dose-2.5 Mrad) at ISOMED, B.A.R.C., Mumbai. After irradiation the implants were evaluated to detect any physicochemical changes. Tests carried out included test for sterility as per I.P. 85, test for colour, odour, visual appearance, uniformity of weight, diameter, thickness, drug content and *in vitro* release.

In vivo studies:

Acute toxicity of sterilised implants was evaluated in mice. Albino mice (19-22 g) were implanted with a single implant on the dorsal side. Groups of 6 mice were used for the study. Control group comprised of 6 mice each implanted with a single unsterilised implant. The implanted mice were observed for a period of 15 days for lethality and abnormal behavior, if any.

Antimalarial efficacy studies were carried out on *P. berghei.* infected male albino mice (18-20 g). The antimalarial efficacy was studied at 2 dose levels-1) 2 implants equivalent to 350 mg/kg and 2) 3 implants equivalent to 500 mg/kg, were implanted subcutaneously on the dorsal side of anaesthetised mice. Groups of 6 mice were used in each case. Four hours later, each of the treated mice was injected intraperitonially with 0.1 ml of parasite stock solution in citrated saline containing $1x10^6$ parasites/500 µl. The parasite was maintained in mice by regular blood passaging. A control group of untreated mice also received a similar dose of infection. The mice were observed daily for development of malaria, any abnormal reactions and lethality. Every week blood smears were prepared and stained to check parasitaemia. The efficacy of the formulations and their long term activity was assessed by determining the mean survival time (MST).

RESULTS AND DISCUSSION

Implants using 10% w/v as EC concentration and 1:1, CQP to EC ratio (Batch A) did not have sufficient plasticity and uniformity of drug distribution. Batch B implants which had a higher EC concentration (15%w/v) but the same drug to polymer ratio were found to have sufficient plasticity and uniformity. Also implants of batch C,D,E, showed uniform distribution of the drug and had required plasticity. Batch D was prepared using 40% w/w PEG 6000 to see if it improves the plasticity of implants. However, no improvement was seen in terms of appearance and plasticity. Air dried blank films of Batch F (With 20% stearic acid) showed nonuniformity in appearance probably because of precipitation of stearic acid resulting in mottled appearance on the surface of the films. In the case of Batch E (with 10% stearic acid), stearic acid tended to recrystallise however the recrystallised stearic acid was found to be uniformly distributed probably due to the high viscosity of the system and these implants appeared smooth and uniform, unlike those of Batch F. Implants of Batch A and F, because of the problems associated with them were not investigated further. The implants of Batch B, C, D and E were obtained as circular, opaque, white discs with smooth lower surface (attached to the release liner) and a relatively rough upper surface. The physical properties and the drug content of implants of Batch B, C, D and E are given in Table 2. The *in vitro* release profiles of uncoated CQP and the implants are shown in Fig. 1. From the release profile it can be seen that implants of Batch B released almost 90% of the drug in the first hour and the release continued only for a period of 24 h. In case of batch C and D implants, about 67% of the drug was released in the first hour. No significant difference in release was seen in

Table 2 : PHYSICAL PROPERTIES AND DRUG CONTENT OF IMPLANTS

Batch	Weight range (mg)	Thickness range (mm)	Diameter range (mm)	Drug content (% w/w)
B	5.88-7.09	0.22-0.24	5.44-5.46	82.31-93.47
C	5.80-6.98	0.22-0.25	5.45-5.48	80.46-91.89
D	6.99-7.63	0.23-0.25	5.44-5.46	75.93-88.56
E	8.19-10.05	0.30-0.33	5.44-5.47	72.65-87.13

Table 3 : EFFECT OF RADIATION STERILIZATION ON CQP IMPLANTS

Characteristic	Unsterilized implants	Sterilized implants
Visual appearance	opaque white	off white tinge
Weight (mg)		
mean±s.d.	9.25±0.69	9.59±0.95
% c.v.	7.53	10.08
Thickness (mm)		
mean±s.d.	0.32±0.01	0.345±0.02
% c.v.	4.79	7.27
Diameter (mm)		
mean±s.d.	5.46±0.03	5.48±0.01
% c.v.	0.49	0.43
Drug Content (% w/w)		
mean±s.d.	82.58±4.61	84.58±5.21
% c.v.	5.21	6.63

Batch E implants were sterilized by gamma radiation (2.5 Mrad) and physicochemical properties of these samples were compared with unsterilized implants.

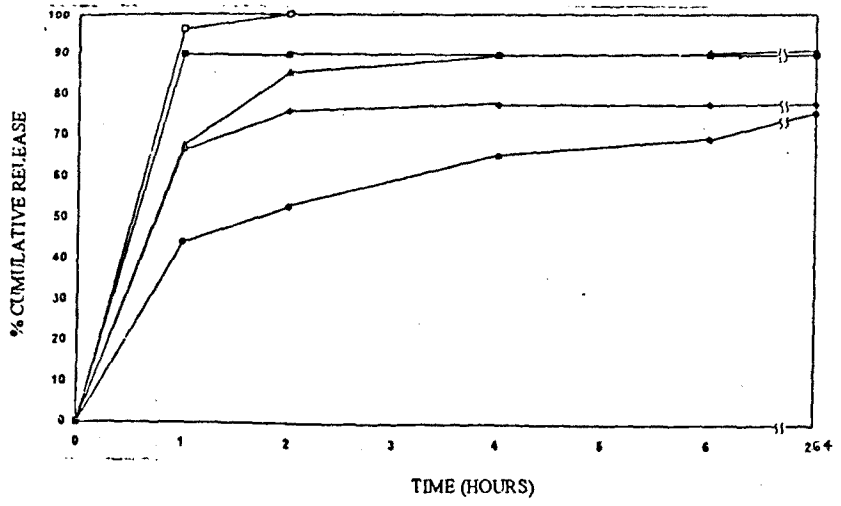

Fig. 1 : *In vitro* drug release profile of CQP from implants using EC as polymer

Drug release protile from C&P implants was assessed in pH 7.4 buffered saline, for a period of 11 days. —⊟—Uncoated drug —■— Batch B, —◆— Batch C, —▲— Batch D, —●— Batch E

implants of batches C and D inspite of addition of 40% w/w PEG 6000 in Batch D, which may act as channeling agent and cause faster release of the drug. Batch E implants which contain stearic acid as release retardant showed a slightly more retardant effect as compared to all other batches and hence were subjected to sterilization and *in vivo* studies.

The rapid release of the drug from the implants could be attributed to the amount of EC being insufficient to retard the release of freely water soluble CQP and/or the punching of bigger discs to obtain implants may lead to the rupture of the EC matrix, particularly at the edges of the implants which may facilitate the leaching of the drug into the dissolution medium. Attempts were made to increase the concentration of EC implants to further retard the drug release and improve the mechanical strength of the implants. However EC solutions in toluene with concentrations greater than 15% w/v were found to be too viscous for casting of films.

On sterilization the implants showed slight discoloration to an off-white tinge which may be due to surface changes that may take place as a result of irradiation[13]. No significant change was found in physical properties and drug content as shown in Table 3 and *in vitro* release of sterilized implant, when compared with unsterilized samples. The implants were found to comply with the test for sterility. In acute toxicity studies no death or abnormal reactions were seen in mice implanted subcutaneously with sterilized implants.

In order to determine the antimalarial efficacy of *P. berghei* infected albino mice, 2 implants (350 mg/Kg) and 3 implants (500 mg/kg) were implanted subcutaneously in groups of 6 mice respectively. Untreated control mice used in antimalarial efficacy studies showed a MST of 10 days, whereas those implanted with drug loaded implants showed a MST of 18 days. Thus the *in vivo* studies indicate that there is an increase in MST of infected mice by 8 days. To assess the safety of high doses employed in implants, groups of 6 mice were injected subcutaneously with 450 mg/Kg of uncoated drug. All the mice exhibited convulsions and death ensued in a period of 10 to 15 minutes. Such toxicity were not seen in mice treated with similar dose of implants.

Thus the *in vivo* studies indicate that the sustained effect lasts for 8 days, however this degree of retardation is not sufficient as required by WHO. Hence further work in this area of research is required to explore the possibility of using higher concentrations of polymers with greater retardant effect, or the use of CQ base which has lower solubility than CQP, in order to achieve the WHO recommendations for prophylaxis.

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REFERENCES

1. Panisko, D.M. and, Keystone, J.S., **Drugs**, 1990, 39, 160.
2. Keystone, J.S., **Drugs,** 1990, 39, 337.
3. Report of a WHO Scientific Group in Advances in malaria chemotherapy, WHO Technical Report Series, 711, Geneva, 1984, 144.
4. Power, K.G., **J. Parasitol.**, 1965, 51 Supplement, 53.
5. Judge, B.M. Howells, R.E. Graham, N.B. and Mc Neill M.E., **Ann. Trop. Med. Parasitol,** 1981, 75, 511.
6. Menon M.D. and Bhalla H.L., in **Proc. Int. Symp. Control. Rel. Bioact. Mater.,** Controlled Release Society, Inc., 193, 20.
7. White N.J., Watt, G., Bergqvist, Y., and Njelesani, E.K., **J. Infec. Dis.**, 1987, 155, 192.
8. White, N.J., Miller, K.B., Churchill, F.C., Berry, C., Brown, J., Williams, S.B., and Greenwood, B.M., **New Engl. J. Med.**, 1988, 319, 1493.
9. Hecquet, B., Fourier, C., Cappelaere, P. and Depadt, G., **J. Pharm. Pharmacol.** 1984, 36, 803.
10. Gurny, R., Simmons, S.P., Banker, G.S., Meeker, R.J. and Myers, R.D., **Pharm. Acta Helv.** 1979, 54, 349.
11. Karunakar B. and Udupa. N., **India. J. Pharm. Sci.** 1993, 55, 68.
12. Tsakala, M., Gillard, J., Roland, M., Chabot, F. and Vert, M **J. Control Release,** 1988, 5, 233.
13. Gopal, N.G.S., **Radiat. Phys. Chem.**, 1978, 12, 35.