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Dose-dependent relationship of polymeric hydrogels on motility and vitality of human spermatozoa *in vitro*

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Alteration in motility and vitality of human spermatozoa treated with various polymeric hydrogels *in vitro* has been studied. Different copolymers of acrylic acid-co-methylmethacrylate, poly (AA-co-MMA); acrylic acid-co-butylacrylate, poly (AA-co-BA); Itaconic acid-co-methylmethacrylate, poly (IA-co-MMA); styrene maleic anhydride, poly (SMA) and homo-polymers of polyacrylic acid, poly (AA); poly methylmethacrylate, poly (MMA) were taken. Of all the polymers studied, the following three groups of water-insoluble polymers, namely poly(IA-co-MMA), poly(AA-co-MMA) and poly (AA-co-BA) proved to be strong inhibitors of spermatozoa motility and vitality. The homopolymer of acrylic acid which is water soluble, also exhibited strong inhibitory action. Poly (MMA) did not show any such effects.

1. Introduction

Rapidly growing population numbers call for a concerted effort towards development of improved methods of contraception. Most current techniques attempt to alter the female system and thereby obtain contraceptive action. More recently research has been directed towards affecting the spermatozoa both within the male reproductive tract as well as in the female system. Guha *et al.* have demonstrated the effectiveness of polystyrene maleic anhydride (SMA) in the lumen of vas deferens [1, 2] and the technique has undergone Phase-I Clinical Trials [3]. A number of vaginal contraceptives which destroy the spermatozoa but do not affect the female reproductive system *per se* are already in use and some other compounds are under investigation [4–11]. Some of the polymers used in male as well as in female systems have been found to be effective. Still there is need for other formulations which can act on the spermatozoa rapidly with a complete inhibitory effect. Singh *et al.* [12] have investigated polystyrene maleic anhydride, poly (SMA); polystyrene maleic acid, polyhydroxy ethyl methacrylate-co-methacrylic acid, poly (HEMA-co-MAC); polyhydroxy ethyl methacrylate and poly methacrylic acid. The criteria for assessment was motility. Considerably reduced motility was taken as indicative of loss of functional capabilities of spermatozoa. Investigations carried out indicate that motility loss cannot alone be taken as the criteria because an immotile spermatozoa may be live and may recover motility at a later stage during transit in the male and female reproductive system. A parameter termed vitality, which is the ratio of the number of live spermatozoa to dead spermatozoa, is required to be taken into account also. The present research has therefore taken both motility and vitality into consideration. A lacuna noted in respect of the earlier study is that an adequate control over the quantitative relationship between the amount of the polymer and the volume of sample treated as well as the number of spermatozoa in the sample has not been considered. If the action is by the pH-lowering effect, the net buffering capacity of the sample treated is obviously important. Different volumes of the sample will have different net buffering capacity and therefore the polymer dose–effect correlation on spermatozoa will vary. Also a smaller number of spermatozoa exposed to a certain polymer mass will have greater functional inhibition than a larger number of spermatozoa exposed to the same mass of polymer. In order to permit objective assessment of the result, the present study observed proper control over the sample volume treated and the best practically achievable control over the spermatozoa number. A strict control over the number is not feasible. Further limitations of earlier reported studies reveal that dose dependence has not been taken into account. As the results presented later in this paper show, the time course of action is highly dose dependent, with the extent of variation in respect to dosage varying from polymer to polymer. Hence the study reported here gives data for multiple dosage of each of the polymers, which have a greater inhibitory action, than the polymers earlier considered.

2. Materials and methods

Several methods of synthesis of these polymers are discussed in the literature. Copolymers of itaconic

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acid and methylmethacrylate have been synthesized by emulsion [13] and solution polymerization [14, 15]. Methyl methacrylate and acrylic acid has been synthesized by charge–transfer copolymerization, solution and bulk polymerization [16–19]. Free radical copolymerization of styrene with maleic anhydrides has been studied by various workers [20, 21] using azobisisobutronitrile and benzoyl peroxide as initiators in bulk as well as in various solvents. The synthesis of SMA using Co^{60} radiation in bulk as well as in solution is also reported [22]. Emulsion polymerization of acrylic acid-co-butylacrylate has been reported [23–25] for making adhesive films. The polymers used here have been synthesized by a γ-irradiation technique as well as by solution polymerization. The method of synthesis of various polymers is given below.

2.1. Synthesis of acrylic acid-co-methyl methacrylate (Group I)

Acrylic acid-co-methyl methacrylate was synthesized by Co^{60} gamma irradiation technique. The radiation polymerization was carried out in standard joint Corning tubes of 12 × 3 cm size. Different percentages of monomers were mixed (Table I) solvent × 6 ml v/v. Nitrogen gas was bubbled through the tubes for a period of 5 min each. The tubes were sealed immediately and subjected to irradiation. A total dose of 0.26 Mrad at dose rate of 28 rad/s was given. After irradiation, the polymer so formed was precipitated with hexane. The precipitate was kept in distilled water overnight with repeated washings of distilled water to remove any homopolymer of acrylic acid. This was followed by refluxing with 1,2-dichloroethane for 8 h to remove the last traces of methyl methacrylate. The copolymer was subjected to vacuum drying over calcium chloride.

2.2 Synthesis of acrylic acid-co-butyl acrylate (Group II)

The synthesis was also carried by γ-irradiation technique keeping all the reaction conditions similar to that of AA-co-MMA. The copolymer was purified by refluxing with hot water to remove homopolymer of acrylic acid. It was then vacuum dried and refluxed with 1,2-dichloroethane for 8 h to remove homopolymer of butylacrylate so formed. The final polymer was stored over calcium chloride.

TABLE I Ratio of various monomers for preparation of copolymers

Feeding ratios of monomers					
Group I		Group II		Group III	
AA%	MMA%	AA%	BA%	IA%	MMA%
100	0	50	50	100	0
75	25	50	25	0	0
25	75	75	25	50	25
0	100	—	—	75	25
solvent used		Solvent used		Solvent used	
Butyl acetate		Ethyl acetate		Dioxane	
(1:10 w/v)		(1:10 w/v)		(1:6 w/v)	

Total dose 0.26 Mrad, dose rate 28 rad/s

2.3. Synthesis of itaconic acid-co-methyl-meth acrylate (Group III)

This polymer could not be synthesized by γ-irradiation technique, hence was synthesized by solution polymerization which was carried out in a round-bottom flask of 250 ml capacity over a water bath at 70 °C using 1% benzoyl peroxide as radical initiator. The solvent used for the reaction was dioxane (1:6 ml v/v). The polymer so formed was precipitated from methanol-ethyl acetate (1:1 v/v). Final purification was carried out with distilled water and 1,2-dichloroethane.

2.4. Synthesis of styrene maleic anhydride

Synthesis of styrene maleic anhydride was carried out according to the procedure reported earlier [26].

2.5. *In vitro* assessment of motility and vitality

To evaluate the effects of polymer exposure on spermatozoa motility and vitality, semen samples were obtained from normal healthy donors by masturbation in the laboratory. Only samples with normal semen characteristics were used in the studies [27, 28]. Ejaculates were allowed to liquefy for 30 min at 37 °C. Sperm suspensions were prepared by using wash and swim-up procedure, at approximate concentration of 20–25 × 10^6/ml, with Ringer-glucose buffer (pH 7.4). To the diluted semen (1 ml) was added 0.003, 0.009, 0.015 and 0.030 g of each polymer. The treated samples were incubated at 37 °C in a CO_2:O_2 incubator for periods of 15, 30, 45, 60, 75, 90 and 120 min. A control was also run wherein no polymer was added to the semen sample. At the end of each time interval, aliquots of 0.02 ml were placed on microscopic slides and observed under a light microscope at a magnification of 40. One hundred spermatozoa were observed in every preparation; spermatozoa were classified as motile or nonmotile depending upon whether any flagellar movement were seen. Percentage vitality of control and treated spermatozoa was evaluated by supravital staining (1% eosin and 10% nigrosin) to differentiate between dead and live spermatozoa [29].

3. Results and discussion

Figs 1 to 12 show the alterations on the motility and vitality patterns of human spermatozoa exposed to various synthesized polymers *in vitro*. Three polymer series, namely poly(AA-co-MMA), poly(AA-co-BA) and poly(IA-co-MMA) were subjected to preliminary screening. Effective polymers from each group and poly(SMA) were taken for the dose-dependent relationship. It is evident from Fig. 1 that poly(AA-co-MMA) synthesized in the molar ratio 3:1 and

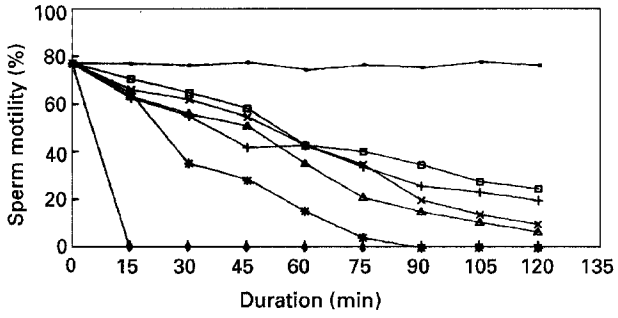

Figure 1 Changes in human spermatozoa motility after treatment with various molar ratios of poly (AA-co-MMA), poly (AA) and poly (MMA) *in vitro* at different treatment intervals (—●— control; —|— AA-co-MMA (1:1); —*— AA-co-MMA (3:1); —□— PMMA; —×— AA-co-MMA; —◆— PAR; —▲—AA-co-MMA (1:3)).

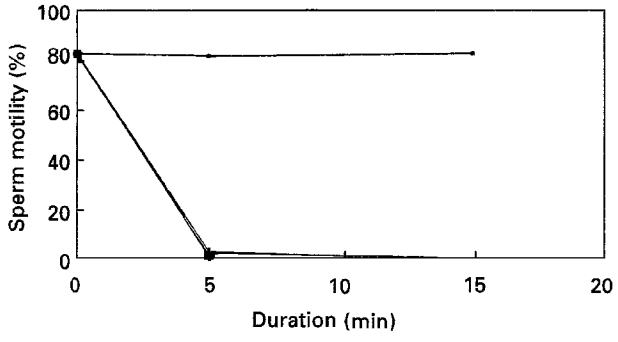

Figure 2 Alterations in sperm motility *in vitro* treated with poly (AA-co-MMA) in the molar ratio of 3:1 at different treatment intervals (—●— control; —|— 0.003 g; —*— 0.009 g; —□— 0.015 g; —×— 0.03 g).

poly(AA) showed strong inhibitory action on spermatozoa motility while other polymers exhibited more moderate effects. On account of this preliminary observation further studies on poly(AA-co-MMA) 1:1 and 1:3 were not conducted. Fig. 2 shows that during treatment with high does, of 0.009, 0.015 and 0.030 g of poly(AA-co-MMA) (3:1 M) all the spermatozoa were rendered immotile within 5 min of polymer exposure, whereas at a low dose of 0.003 g it took 15 min to obtain complete immotility as compared to the control. Similar effects were observed with poly(AA) treatment at various doses (Fig. 3). Poly(AA) is found to be completely soluble in aqueous medium while poly(AA-co-MMA) (3:1 M) is partially soluble. Figs 4 and 5 depict the spermatozoa vitality during corresponding intervals. It is evident that at higher doses when complete loss of spermatozoa motility was observed after 5 min of drug exposure, 15–30% of spermatozoa were still found live. The data highlight the fact that the spermatozoa exposure to the polymer does not kill them initially.

From poly(AA-co-BA) series, the polymer of molar ratio 3:1 M proved to be effective (Fig. 6) while at other ratios of 2:1 and 1:1, it exhibited a less significant inhibitory action. These polymers were found to be insoluble in aqueous medium therefore all were subjected to dose-dependent screening, considering the utility of these as intravasal drug depot. Complete

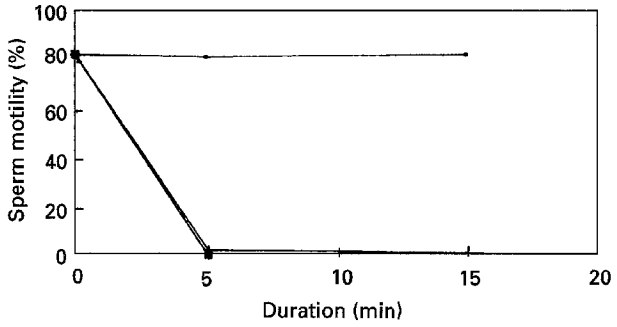

Figure 3 Alterations in the motility patterns of human spermatozoa *in vitro* treated with poly(AA) at various treatment intervals (—●— control; —|— 0.003 g; —*— 0.009 g; —□— 0.015 g; —×— 0.03 g).

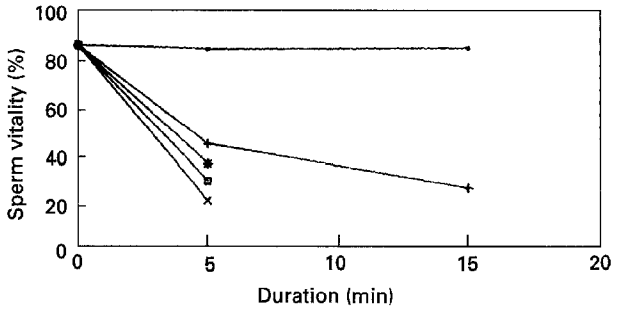

Figure 4 Illustration showing changes in the spermatozoa vitality of human spermatozoa treated with poly (AA-co-MMA) (3:1 M) *in vitro* at various treatment intervals (—●— control; —|— 0.003 g; —*— 0.009 g; —□— 0.015 g; —×— 0.03 g).

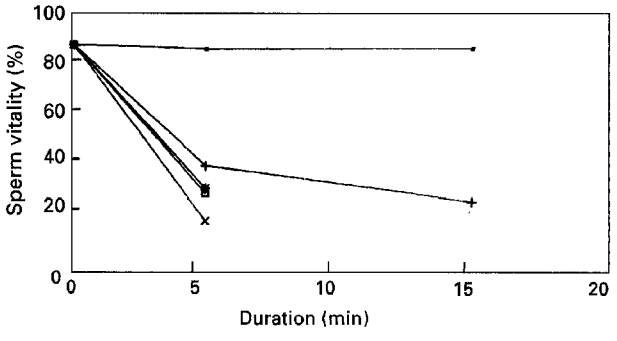

Figure 5 Alterations in spermatozoa vitality *in vitro* after exposure to poly (AA) at different durations (—●— control; —|— 0.003 g; —*— 0.009 g; —□— 0.015 g; —×— 0.03 g).

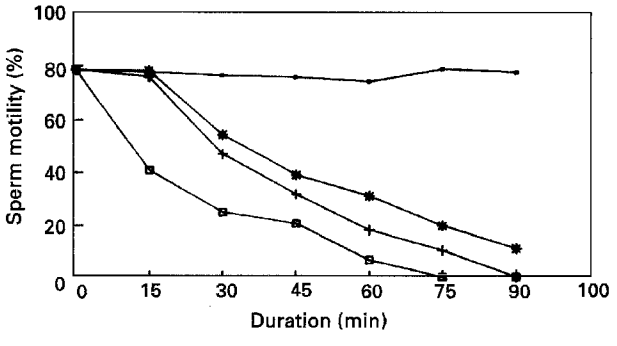

Figure 6 Alterations in spermatozoa motility *in vitro* treated with various molar ratios of polymer series—poly (AA-co-BA) at different treatment intervals (—●— control; —|— AA-co-BA (1:1); —*— AA-co-BA (2:1); —□— AA-co-BA (3:1)).

loss of spermatozoa motility was observed within 5 min of 3:1 M poly(AA-co-BA) treatment with all the doses investigated (Fig. 7) as compared to control. Fig. 8 explains the spermatozoa vitality at different

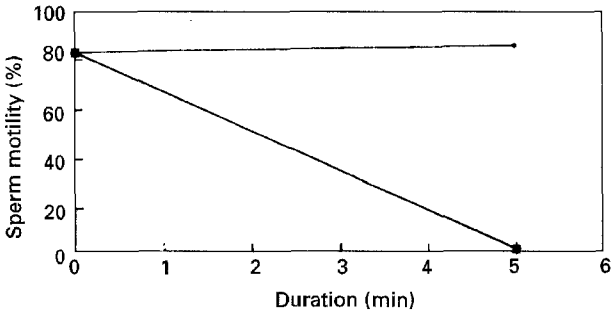

Figure 7 Alterations in motility patterns of human spermatozoa *in vitro* treated with poly (AA-co-BA) in the molar ratio of 3:1 at various treatment intervals (—●— control; —|— 0.003 g; —*— 0.009 g; —□— 0.015 g; —×— 0.03 g).

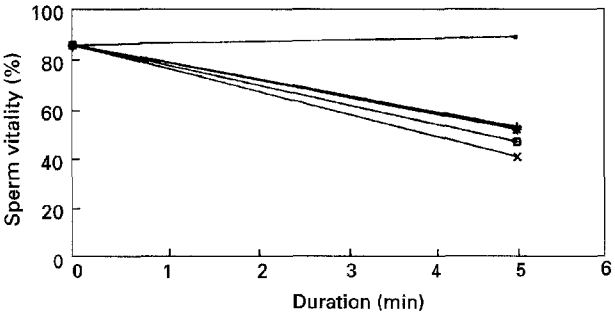

Figure 8 Alterations in the percentage spermatozoa vitality *in vitro* treated with poly (AA-co-BA) in the molar ratio of 3:1 at various treatment intervals (—●— control; —|— 0.003 g; —*— 0.009 g; —□— 0.015 g; —×— 0.03 g).

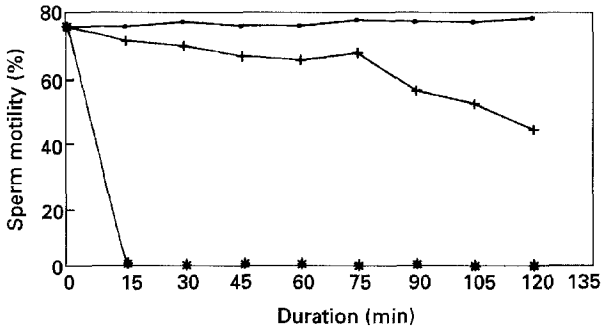

Figure 9 Alteration in the motility patterns of human spermatozoa treated with 3:1 molar ratios of poly (IA-co-MMA) and with poly (MMA) *in vitro* at different treatment durations (—●— control; —|— PMMA; —*— IA-CO-MMA (3:1)).

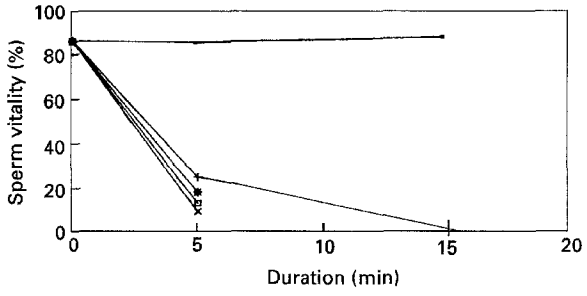

Figure 10 Alteration in the vitality patterns of human spermatozoa *in vitro* treated with poly (IA-co-MMA) at different treatment intervals (—●— control; —|— 0.003 g; —*— 0.009 g; —□— 0.015 g; —×— 0.03 g).

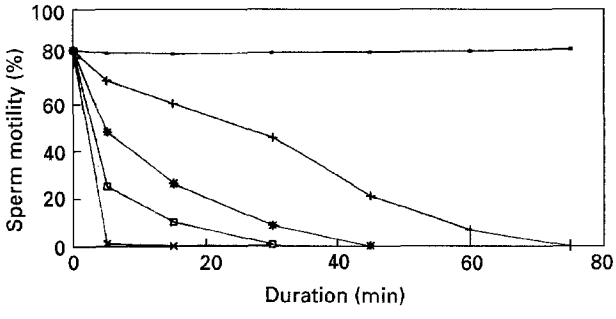

Figure 11 Alterations in the motility patterns of human spermatozoa *in vitro* after treatment with poly (SMA) during various treatment intervals (—●— control; —|— 0.003 g; —*— 0.009 g; —□— 0.015 g; —×— 0.03 g).

treatment intervals. After 5 min of treatment for all the doses taken, complete loss of motility was obtained, while 35, 42, 44 and 45% of spermatozoa at 3,9,15 and 30 mg doses, respectively, did not die. The other two polymers synthesized in the molar ratios 2:1 and 1:1 showed similar motility and vitality patterns.

In the third series of polymers two ratios, 2:1 and 3:1, of poly (IA-co-MMA) were taken, and poly (MMA) was also studied. With both ratios poly (IA-co-MMA) exhibited similar effects on spermatozoa motility patterns whereas poly (MMA) did not show any significant inhibition of spermatozoa motility (Fig. 9). Ply (IA-co-MMA) at 3:1 M ratio proved to be the most potent among all the polymers studied. At all dose levels complete loss of spermatozoa motility was evident within 5 min of polymer exposure, whereas 8–25% spermatozoa vitality was evident at this stage (Fig. 10).

Figs 11 and 12 present details of alterations to the inhibition of spermatozoa motility; complete loss of spermatozoa motility was obtained after 15 min of 0.030 g polymer exposure whereas such an effect was observed after 30, 45 and 75 min treatment at 0.003, 0.009 and 0.015 g doses, respectively (Fig. 11). During corresponding intervals data on the vitality of the spermatozoa depicts that high doses of the polymer first affects the motility, and that then spermatozoa gradually die in the course of prolonged treatment at various doses (Fig. 12).

Decrease in spermatozoa motility may be explained through the pH-lowering effect of the medium [30]. Inhibition of spermatozoa motility and vitality may also be due to: (i) uncoupling of oxidative phosphorylation at the mitochondrial level; (ii) inhibition of spermatozoa specific isoenzyme LDH-X; and (iii) impairment of the ATPase activity [31–35].

For use as an intra vas deferens contraceptive a polymer with long-term depot-forming characteristic is required. Only a non-water-soluble polymer can serve this purpose. On the other hand in the case of a vaginal contraceptive a water-soluble polymer is to be preferred, but non-water-soluble polymer can also be used. Therefore in the present study two categories of polymers have been investigated, a water-insoluble class represented by poly (AA-Co-BA), poly (AA-Co-MMA), poly (IA-Co-MMA), and a water-soluble class represented by poly acrylic acid.

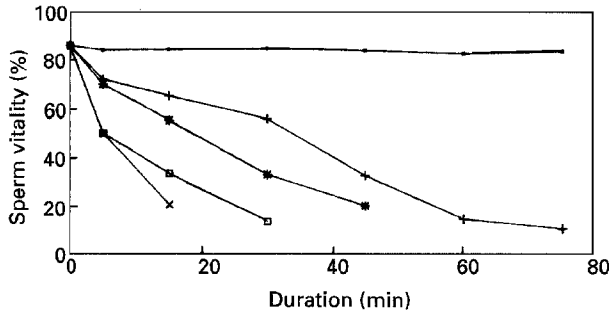

Figure 12 Alterations in spermatozoa vitality *in vitro* treated with poly (SMA) at different treatment intervals (—●— control; — — 0.003 g; —*— 0.009 g; —□— 0.015 g; — — 0.03 g).

The results from the present study suggest that nonsoluble polymers can be considered as potential candidates for the development of an intravasal contraceptive whereas water-soluble polymers are suitable for the development of a vaginal contraceptive.

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