Ceylon J. Sci., Biol. Sci. Vol. 16 (1&2) December 1983

EFFECT OF POLYPHLORETIN PHOSPHATE (A PROSTAGLANDIN RECEPTOR-BLOCKING DRUG) ON FERTILITY OF MALE RATS.

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ABSTRACT

No significant suppression in fertility and libido was evident by implanting Silastic rods containing 25% and 50% polyphloretin phosphate, a prostonoid receptor-blocking drug, adjacent to the epididymis of rats. The treatment neither produced any significant deterioration of health nor development of undesirable side effects. Possible reasons for the failure of polyphloretin phosphate to affect fertility are discussed.

INTRODUCTION

Prostaglandins are reported to be present in the epididymis of rat (Johnson & Ellis 1977; Gerozissis & Dray, 1977; Kelly 1981). A functional role for prostaglandins in the regulation of orgasmic contraction of the epididymis at ejaculation (Marley & Smith 1974 (a), 1974 (b)) and in the maturation process of sperm within it (Poulos, Voglmayr & White 1973) have been suggested. It should therefore be possible to reduce fertility of males by treatment with prostaglandin receptor-blocking drugs.

The aim of the present study is to explore this possibility in rats by administering polyphloretin phosphate, a prostaglandin receptor-blocking drugs (Eakins & Karison 1970; Horton 1976), locally to the epididymis via a slow-release drug delivery system made from medical Silicone elastomer.

METHODS

Animals Adult laboratory-bred albino rats of proven fertility weiging 200-300 g were used. They were housed in a well-ventilated animal house at a temperature of 28-30 ^{0}C with a natural photoperiod (about 12 h light and 12 h dark daily). All rats received food (rat pellets and green leaves) and tap water ad libitum.

Ceylon J. Sci., Biol. Sci. Vol. 16 (1&2) December 1983

Construction of slow-release drug delivery system

Rods containing 25% and 50% polyphloretin phosphate respectively were made by mixing known weights of powdered polyphloretin phosphate (Leo) and polysiloxane polymer (Silastic 382, Medical Grade Elastomer, Dow Corning Ltd, U.S.A.) using a pestle and mortar (Ratnasooriya, Gilmore & Wadsworth 1980). Completed rods were 3 - 5 mm in diameter and 8 - 9 mm in length. Control rods consisting entirely of Silastic were also manufactured. Polyphloretin phosphate and control rods were stored at 10 - 15 ^{0}C in brown glass vials until insertion in animals.

Insertions of rods and fertility trials

Insertion of rods was performed using aseptic precautions under mild ether anaesthesia. A rod containing 25% polyphloretin phosphate (6 rats), 50% polyphloretin phosphate (12 rats) or a drug-free rod (12 rats) was placed adjacent to each epididymis via an incision made in the scrotal sac and in the tunica vaginalis of each side as described by Ratnasooriya et al. (1980). The day of surgery was designated as day 0.

Libido and fertility of the treated or control animals were tested at day 3 and day 7 and then at weekly intervals by overnight pairing of each male with a pro-oestrous female of normal oestrous cycle. The sexual behaviour pattern of the male towards the female was noted after pairing for 2 - 4h.

Vaginal smears were taken at 6.00 a.m. on the following morning to check insemination, and rough estimates of motility and numbers of ejaculated sperm were also made. If spermatozoa were absent, daily smearing was undertaken to check for pseudopregnancy. The mated females were laporatomized 10 - 12 days after pairing and the numbers of embryos present were counted. The results were analysed with Mann-Whitney U test (Siegel 1956): p values of less than 0.05 were considered statistically significant.

RESULTS

During the study, one animal fitted with 25% polyphloretin phosphate epidiymal rods (male 4) died on day 8. The cause of death was unknown; however, there was no apparent relationship to the treatment given as autopsy revealed absence of any drug related signs and lesions representing gastrointestinal intolerence. Autopsy of this animal also showed that the rods were in position. The general health of the remaining animals appeared to be normal.

Ceylon J. Sci., Biol. Sci. Vol. 16 (1&2) December 1983

The results of the fertility trials are depicted in Table 1. Fertility was not significantly reduced in animals with 25% or 50% polyphloretin phosphate rods compared to those with drug free rods. However, male 45 and 4 of the treated group became subfertile on day 3 and day 7 respectively. The subfertility in male 45 was temporary since fertility was restored to normal levels from subsequent mating (see Table 1). In the case of male 4, subfertility may have resulted from deterioration of its general physical condition at the time of mating since this animal died on the following day. Vaginal smears of the females mated with males fitted with either drug free rods or polyphloretin rods had apparently normal numbers of motile sperm. Libido and potency remained unaltered as judged by normal premating and mating behaviour.

DISCUSSION

The results of the current study show that the chronic local application of polyphloretin phosphate, a prostaglandin receptor-blocking drug, caused neither a significant impairment in fertility nor in the ability of the animals to court or to mate. Since male libido is androgen-dependent(Neuman 1977), its preservation without any detectable alteration following the application of 25% or 50% polyphloretin phosphate suggests that the androgen output from the testes is not depressed. However, a lack of a siginificant depression in fertility observed here is rather enigmatic in view of the reported prostaglandin receptor blocking activity of the drug (Eakins & Karison 1970; Horton 1976) and of the roles that prostaglandins are claimed to play in maintaining normal reporoductive function (Bartke 1976) and fertility of males (Bygdeman. Fredricsson, Svanborg & Samulsson 1970)

One possible explanation is that insufficient drug is being released from the Silastic formulation to antagonise fairly high concentrations of endogenous prostaglandins available in the epididymis and vas deferens (Johnson & Ellis 1977). It has been found that polyphloretin phosphate has weak potency in blocking the prostonoid receptors (Bennet, Eley & Stockley 1976). However, by employing the present form of drug/Silastic formulation it is not practically feasible to augment drug concentration further (Wadsworth & Ratnasooriya 1981). An alternative conclusion is that the main prostaglandin that is concerned with fertility of male rats may be acting through a specific prostonoid receptor which is not capable of being blocked by polyphloretin phosphate. Evidence has now accumulated to indicate that prostonoid receptors are composed of a heterogeneous population consisting of

Ceylon J. Sci., Biol. Sci. Vol. 16 (1&2) December 1983

at least three or four types (Copas, Gardiner & Wilson 1981 (a), 1981 (b); Coleman, Feniuk & Kennedy 1981). and the binding affinities of these different receptor types vary with the drug used. Furthermore, a variety of prostaglandins occur in the male reproductive system (Bartke 1976). A third explanation is that the failure of polyphloretin phosphate in reducing fertility may have resulted from the absence of prostonoid receptors, or binding sites, within the epididymis. A positive correlation between human sperm motility and prostaglandins has been established (Cohen, Colin, Golimbus & Hotchkiss 1977) inspite of lack of prostonoid receptors (Schlegel, Rotermund, Farber & Nieschlay 1981) indicating that prostaglandins are not mediating their actions in sperm through receptors. This may well be the case with the epididymis too. These possibilities were however, not explored since the drug had no significant effect on fertility.

Thus, the failure of the prostonoid receptor-blocking drug, polyphloretin phosphate, to affect fertility in the male rat indicates that this drug cannot be used as a potential male fertility regulating drug which acts via the epididymis. However, such failure as reported here should not dissuade further investigation of other prostonoid receptor-blocking drugs as potential male contraceptive agents.

ACKNOWELDGEMENTS

We thank Dr. R.M. Wadsworth, Department of Physiology and Pharmacology, Strathclyde University, Glasgow, United Kingdom, for critically reviewing this manuscript and Dr. Fex, Aktlebolaget Leo, Sweden, for the generous gift of polyphloretin phosphate. This work was financed by the National Science Council of Sri Lanka Grant No 80/57.

REFERENCES

Bartke, A. (1976). Prostaglandin and function of the male reproductive system. In:Regulatory mechanisms of male reproductive physiology, Eds. Spilman, C.H.,Lobl, T.J. & Kirton, K.T. P. 79-96. Excerpta Medica: Amsterdam.

Bennett, A., Eley, K.G. & Stockley, H.L. (1976). Inhibition of peristalsis in guinea-pig isolated ileum and colon by drugs that block prostaglandin synthesis. Br. J. Pharmac. 57: 335-340.

Ceylon J. Sci., Biol. Sci. Vol. 16 (1&2) December 1983

Bygdeman, M., Fredricsson, B., Svanborg, K. & Samulsson, B. (1970). The relation between fertility and prostaglandin content of seminal fluid in man. Fert. Steril. 21:622-629.

Cohen, M.S., Colin, M.J., Golimbus, M. & Hotchkiss, R.S. (1977). The effects of prostaglandins in sperm motility. Fert. Steril. 28(1): 78-85.

Coleman, R.A., Feniuk, L. & Kennedy, I. (1981). A study of the prostonoid receptors mediating bronchoconstriction in the anaesthetised guinea-pig and dog. Br. J. Pharmac. 74: 913-914.

Copas, J.L., Gardiner, P.J. & Wilson, S.A. (1981 a). TR 4979, a selective 'ψ' prostonoid receptor agonist in the air ways. Br. J. Pharmac. 74: 795.

Copas, J.L., Gardiner, P.J. & Wilson, S.A. (1981 b). The selectivity of TR 4979 for 'ψ' prostonoid receptors. Br. J. Pharmac. 74: 912-913.

Eakins, K.E. & Karison, S.M.M. (1970). A selective antagonist for prostaglandins $F_1\alpha$ and $F_2\alpha$ Life Sci. 9(1): 1-5.

Gerozissis, K. & Dary, F. (1977). Selective and age-dependent changes of prostaglandin E_2 in the epididymis and vas deferens of the rat. J. Reprod. Fert. 50: 113-115.

Horton, E.W. (1976). Prostaglandins. Sci. Prog. Oxford 63: 335-346.

Johnson, J.M. & Ellis, L.C. (1977). The histochemical localization of prostaglandin synthetase activity in reproductive tract of male rat. J. Reprod. Fert. 51: 17-22.

Kelly, R.W. (1981). Prostaglandin synthesis in male and female reproductive tract. J. Reprod. Fert. 62: 293-304.

Marley, P.B. & Smith, C.C. (1974 a). Mating increases the prostaglandin-like content of male and female reproductive tracts in mice. J. Endocr. 61: XXXIV.

Ceylon J. Sci., Biol. Sci. Vol. 16 (1&2) December 1983

Marley, P.B. & Smith, C.C. (1974 b). The source and possible function in fertility of seminal prostaglandin-like material in mouse. Br. J. Pharmac. 52: 114.

Neumann, F. (1977) Pharmacology and potential use of cyproterone acetate. Horm. Metab. Res. 9: 1-13.

Poulos, A., Voglmayr, J.K. & White, I.C. (1973). Phospholipid changes in spermatozoa during passage through the genital tract of bull. Biochim. biophys. Acta 306: 194-202.

Ratnasooriya, W.D., Gilmore, D.P. & Wadsworth, R.M. (1980). Effect of local application of sympathomimetic drugs to the epididymis on fertility in rats. J. Reprod. Fert. 58: 19-25.

Schlegel, W., Rotermund, S., Farber, G. & Nieschlag, E. (1981). The influence of prostaglandins on sperm motility. Prostaglandins 21(1): 87-99.

Siegel, S. (1956). Non-parametric Statistics for Behavioral Sciences. McGraw-Hill, New York.

Wadsworth, R.M. & Ratnasooriya, W.D. (1981 . Method for localized and sustained administration of drugs to the vas deferens of rats. J. Pharmacological Methods. 5: 313-320.

Table 1 The effect of 25% Polyphloretin phosphate on fertility of male rats when applied locally to epididymis in successive breeding trials.

Treatment	Animal No.	Number of embryos: Days after implantations of rods						
		3	7	14	21	28	35	42
Control	10	9	9	9	8	NM	10	8
	11	8	8	10	8	-	8	12
	12	8	8	11	6	12	10	8
	13	5	8	7	7	-	10	7
	14	11	6	8	3	7	11	8
	15	8	8	8	NM	8	-	-
	16	8	7	8	8	8	9	8
	17	8	8	7	8	8	8	9
	20	10	9	11	5	10	8	8
	21	10	10	8	8	8	9	9
	22	8	8	9	9	9	5	8
	24	8	8	10	8	-	8	12
25% Polyphloretin phosphate	4	8	1	-	-	-	-	-
	5	8	9	9	6	NM	8	8
	6	7	4	8	6	6	5	6
	77	9	8	8	6	4	7	8
	8	7	7	7	7	7	8	7
	9	6	8	5	7	-	NM	8

Table 1 Contd.

Treatment	Animal No.	Number of embryos						
		Days after implantation of rods						
		3	7	14	21	28	35	42
Control	36	8	9	9	7	9	9	9
	38	10	8	NM	8	6	-	8
	40	NM	9	8	8	9	9	9
	44	9	9	8	9	9	8	9
	45	2	7	7	6	8	9	9
	46	8	7	9	8	8	8	-
	60	9	10	7	7	8	8	9
	64	10	9	8	NM	8	7	6
	65	9	6	9	9	7	8	-
	67	8	10	8	10	10	8	8
	68	8	7	8	6	10	6	9
	69	8	8	6	9	8	8	8

NM = mating did not take place at this pairing.