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Nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester impair fertility of female rats when given during early pregnancy

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Abstract

The role of nitric oxide (NO) on fertility of female rats was investigated by oral administration of the NO synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) (50 or 100mg/kg/day) to rats from days 1-7 of gestation. L-NAME caused a moderate but significant reduction ($P<0.05$) in number of uterine implants. This antifertility effect was due to an elevation in pre-implantation loss. The inactive stereoisomer D-NAME, on the other hand, had no effect whatsoever on fertiligy. The results suggest that NO is essential for the expression of normal fertility in female rats and NO donors may find applications in the treatment of some forms of female subfertility.

Key Words: N-nitro L-arginine methyl ester (L-NAME), nitric oxide, nitric oxide synthase, fertility, pre-implantation loss.

1. Introduction

Nitric oxide (NO) is distributed almost throughout the female reproductive system [ovary, Jawerbaum *et al.,* (1999); uterine, myometrium, Ai-Hijji *et al.,* (2000); vagina, Ai-Hijji *et al.,* (2000); oocyte-cumulus complexes, Jawerbaum *et al.,* (1999]. NO is also produced by pre implantation embryos (Gouge *et al;* 1998) and placenta (King *et al.,* 1995). Moreover , in the rat, NO production is increased during pregnancy (Nowicki *et al.,* 1997). It has been therefore claimed that NO is important in the female reproductive system (Jawerbaum *et al.,* 1999; Al-Hijji *et al.*, 2000; Gouge *et al.,* 1998) and modulate menstrual bleeding, (Chawalisz and Garfield,

2000); ovulation, (Li *et al.,* 1991); fertilization, (Jawerbaum *et al.,* 1999); endometrial receptivity,(Chawalisz and Garfield 2000), implantation, (Chawalisz and Garfield, 2000); embryonic development, (Gouge *et al.,* 1998) and parturition, (Maki *et a* 1993).

It is well established that prostaglandins (PGS), particularly the PGF_{2x} and PGE generated by uterine and/or embryos are involved in the initiation of blastocyst implantation (Novaro *et al.,* 1996). NO can stimulate cyclooxygenase activity, the enzyme catalysing the production of PGS (Franchi *et al* 1994). Thus, NO can also regulate uterine implantation indirectly via PGS.

Collectively, these observations suggest that NO may play a role in the regulation of female fertility. This can be tested scientifically in the laboratory by using either inhibitors of NO synthase or NO donors. The study reported here in was therefore initiated to assess the effects of NO synthase inhibitor N-nitro-L-arginine methyl ester, L-NAME on fertility of female rats.

2. Materials and methods

Healthy adult crossbred albino rats (males weighing 250-275 g and females weighing 175-225g) of proven fertility were used. All rats were kept under standerdised animal house conditions (temperature 28-31^{0}C, photoperiod approximately 12h natural light per day, 50-55% relative humidity) with free access to pelleted food (Vet House Ltd., Colombo, Sri Lanka.) and tap water.

L-NAME and D-NAME (Sigma Chemical Co., Poole, Dorset, UK) were dissolved in 0.9% NaCI (w/v) to obtain the required concentration (50 or 100mg/kg) in 1 ml of solution.

Pro-oestrous rats (n=48) were selected by vaginal smearing and individually paired with a male rat (between 17.00h and 18.00h). Successful matings were confirmed by the presence of sperm in the vaginal smear (>5x10^{6}/ml) the following morning (between 7.00 and 8.00h), and this designated as day 1 of presumed pregnancy.

Pregnant rats were randomly divided in to 4 equal groups (n=12). The first group was daily orally administered (at 9.00-10.00h) with 50mg/kg of L-NAME, group two with 100mg/kg of L-NAME, group three with 50mg/kg of D-NAME and group 4 with 1 ml of saline, for 7 consecutive days from day 1 of pregnancy.

All rats were observed at least once daily until laparotomy for mortality, general health, gross behavioural changes, overt signs of stress and toxicity, vaginal bleeding and diarrhoea. In addition, food and water intake was noted. At 14 days after mating, the rats were subjected to uterotomy under ether anaesthesia using aseptic precautions to determine the number, viability, size (using vernier calipers) and distribution of fetuses, the number of resorption sites and the gross appearance of the corpora lutea.

The rats were sutured, treated locally and subcutaneously with tetracycline hydrochloride (Astron Ltd., Ratmalana, Sri Lanka) and oxytetracycline (Anglian Nutrition products Co., Hadleigh, UK) respectively, and allowed to recover and deliver. Upon delivery, the number pups born, their viability and gross apearance were recorded.

Based on laporotomy and neonatal data the following reproductive indices were computed: quantal pregnancy =(number pregnant/number mated) x 100; fertility index = (number pregnant/number paired) x 100; implantation index = (total number of implantations/number mated)x 100; pre-implantation loss = [(total number of corpora lutea-total number of implantations)/total number of corpora lutea]x100; post-implantation loss =[(total number of implantations-total number of viable implants)x100; and resorption index = (namber of resorptions/ total implantations) x 100

The data are given as means±SEM. Statistical analyses were made using Mann Whitney U test and G test (for quantal data) as appropriate. Differences with P values less than 0.05 were regarded as significant.

3. Results

During the study, deaths or observable deterioration in health was evident. L-NAME treated animals showed normal food and water intake and body size appeared to be normal. Further, neither dose of L-NAME had any observable effect on alertness, locomotory activity, rearing, face cleaning, self grooming, salivation, fur erection and bulging of eyes. In addition, none of the treated rats showed vaginal redness or bleeding or spontaneous fetal discharges.

At laporotomy, functional hyperaemic corpora lutea were evident in all rats. The foetuses when present were neither aggregated nor pushed towards the cervical end of the uterus. The appearance of the fetuses from the treated group was normal.

Table 1 summarizes the data obtained in the fertility study. None of the fertility or pre-natal developmental parameters was significantly ($P>0.05$) altered by D-NAME. In contrast, both doses of L-NAME significantly ($P<0.05$) reduced the number of uterine implants: lower dose by 42% and higher dose by 26%. However, none of the treated rats became completely sterile. Pre-implantation loss was also markedly and significantly ($P<0.05$) increased following both doses of L-NAME. However, other parameters investigated in L-NAME treated rats were not significantly ($P>0.05$) altered.

4. Discussion

In this study, both the lower dose (50mg/kg/day) and the higher dose (100mg/kg/day) moderately impaired the fertility (in terms of number of uterine implants.) of female rats. This is a novel and important finding which may also have clinical implications in the treatment of female subfertility and urogenital infections in pregnancy (Nowicki *et al.,* 1997). In contrast, profound inhibition in fertility (both in terms of uterine implants and quantal pregnancy)has been reported in male rats treated with L-NAME (Ratnasooriya *et al.,* 2000). L-NAME induced antifertility effect in this study was not dose-related although the lower dose used has been shown to inhibit No formation (Arnal *et al.,* 1993). This may be due to up regulation of NO synthase activity as evident in tunica albuginea of rats (Trinity *et.al.,* 2000). The inactive stereoisomer, D-NAME, had no effects whatsoever on fertility. Thus, the antifertility effect of L-NAME can be attributed to inhibition of NO formation. Absence of any signs of lethargy, behavioural abnormalities or overt signs of toxicity in L-NAME treated rats lends further support to this proposition.

L-NAME treatment caused a marked elevation in pre-implantion loss and had no effect on other reproductive parameters investigated. Thus, this is likely to be the main mechanism of inhibition of fertility by L-NAME. Stress known to increase pre-implantion losses (Ratnasooriya and Wodsworth, 1987) but L-NAME was not stressogenic (absence of fur erection exopthalmia, salivation, or weight loss). Hence, this mechanism is unlikely to be operative here.

In the rat, mating generally occurs between 22.00-24.00h on the day of prooestrous (Bedford, 1974) and fertilization occurs 5.0-6.h following

mating (Bony and Frantis, 1990). In this study, L-NAME was administered 11-12h following mating. Therefore, preimplantation losses are unlikely to be due to interruption in events of fertilization.

On the other hand, L-NAME is likely to elevate pre-implantation losses by interrupting endomatrial receptivity and/or intrauterine implantation process, via inhibition of NO formation: NO is a smooth muscle relaxant and a powerful vasodialator (Novaro *et al.,* 1996) and successful implantation requires an increase in vascular permeability and uterine quiescence at the site of blastocyte implantation (Gouge *et al.,* 1998). Alternatively, there is evidence that NO activates cyclooxygnase enzyme, which produce prostoglandins (PGS) (Franchi *et al.,* 1994) PGE and PGF_{2x} are involved in implantation process (Novaro *et al.*, 1996). Therefore, L-NAME by its ability to inhibit NO formation could suppress PG formation and disrupted the implantation process in consequence: indeed, L-NAME has been shown to decrease PGE production in the rat uterine tissue (Novaro *et al* 1996).

L-NAME, though its NO synthesis inhibition, is known to impede development of pre-implantation embryos (Gouge *et al.,* 1998). Embryos of a critical stage of development are mandatory for proper implantation (Trinity *et al.,*2000). As such, L-NAME could have interfered with implantation process by this mechanism too.

In conclusion, the present finding that L-NAME impairs fertility in female rats suggest that NO is essential for the expression of normal fertility in female rats, as in the males (Ratnasooriya *et al.,* 2000). The results indicate that NO-status may have a bearing on pregnancy of women who are less fertile and in infectious complications during pregnancy (Nowicki *et al.,* 1997).

Table 1-Effects of oral treatment (dayl - 7 of pregnancy) of D-NAME (50mg/kg/day) or L-NAME (50 or 100mg/kg/day) on some fertility parameters of female rats. (mean ±SEM: range in parentheses.)

Parameters	Control	D-NAME	L-NAME	
		50mg/kg/day	50mg/kg/day	100mg/kg /day
Quantal pregnancy(%)	100	100	83.3	100
No. of implantation	9.6±0.6	9.5±0.6	5.6±1.0**	7.1±0.5*
	(7-11)	(9-11)	(3-11)	(6-9)
Implantation index (%)	966.6	950	660	716.6
Pre-implantation loss (%)	20.5±3.5	25.2±2.4	46.8±12.5*	34.4±5.2*
	(9-30)	(15.3-30)	(27.2-58.3	(12.5-50)
Post-implantation loss (%)	0.0	0.0	0.0	0.0
Resorption index (%)	0.0	0.0	0.0	0.0
Gestation index (%)	920	783	660	716
Live birth index (%)	100	100	100	100
Fetal length (mm)	10±0.25	10±0.5	10.1±0.5	9.0±0.5
	(9-11)	(8-11)	(7.9-11)	(7-10)
Fetal survival ration (%)	100	82.45	100	100
Letter index (%)	100	82.45	100	100
Mean number of pups	9.2±0.5	6.6±1.0	7.2±1	7.8±0.5
born	(6-11)	(5-8)	(6-9)	(6-9)

As compared with control;*p<0.05, **P<0.001 (Mann Whitney, U test and G test)

5. References

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