An argument for the past and continued use of pentoxifylline in assisted reproductive technology

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Pentoxifylline was first used within an in-vitro fertilization (IVF) programme before the advent of alternative treatment strategies such as oocyte micromanipulation. Over the years, it has continued to be useful in aiding fertilization in selected IVF cases, with a beneficial effect also being seen in certain cases treated by intrauterine insemination. In both instances, the acrosome reaction to ionophore challenge test appears to have been invaluable in identifying suitable patients. The stimulation of spermatozoa by pentoxifylline should remain a therapeutic option in the treatment of couples with a male factor present. As an adjunct to IVF, it has the advantage of being simpler and less costly to perform compared with micromanipulation. However, its use should be restricted to selected cases, and the merits over and above those of invasive procedures such as intracytoplasmic sperm injection should be discussed with the individual patients. The pretreatment of spermatozoa prior to intrauterine insemination in selected cases gives an alternative therapeutic strategy to those patients not wishing or unable to undertake IVF.

Key words: assisted reproductive technology/IVF/pentoxifylline

Introduction

The failure of oocytes to fertilize can be seen for many couples irrespective of the apparent quality of the male partner's semen (Matson *et al.*, 1989), and this has traditionally been overcome by using donor spermatozoa. However, recent technology has enabled fertilization to be achieved in such cases with the partner's spermatozoa by the micromanipulation of oocytes to reduce the barriers presented to the spermatozoa, using procedures such as partial zona dissection (Cohen *et al.*, 1988), subzonal insemination (Ng *et al.*, 1988) and intracytoplasmic sperm injection (Palermo *et al.*, 1992) to by-pass both the zona pellucida and the vitelline membrane. Nevertheless, an alternative strategy has been to improve the performance of the spermatozoa with the chemical stimulant pentoxifylline, so that zona-intact oocytes may be fertilized (Yovich *et al.*, 1990) and the mechanisms for the selection of the spermatozoon by the oocyte left intact. An argument for the past and continued usefulness of pentoxifylline in the treatment of male factor human infertility is presented here.

Action and clinical application of pentoxifylline

Pentoxifylline (or oxpentifylline as it is often known) is a methylxanthine which acts as a phosphodiesterase inhibitor. Its chemical structure is given in Figure 1. By virtue of its ability to improve perfusion in the impaired microcirculation of peripheral and cerebral vascular beds, it has been administered clinically to patients to improve blood flow in cases of peripheral vascular disease presenting with intermittent claudication (Ward and Clissold, 1987), with a 400 mg sustained slow-release preparation (as is available commercially) resulting in peak blood concentrations of pentoxifylline of ~0.3 μg/ml. The primary mechanism by which pentoxifylline increases blood flow appears to relate to an overall improvement in haemorheological characteristics, such as erythrocyte deformability, blood viscosity, platelet aggregation and plasma fibrinogen concentrations.

As with all licensed drugs, pentoxifylline (Trental; Hoechst) has undergone rigorous toxicity and teratological testing. Lethal toxicity in common

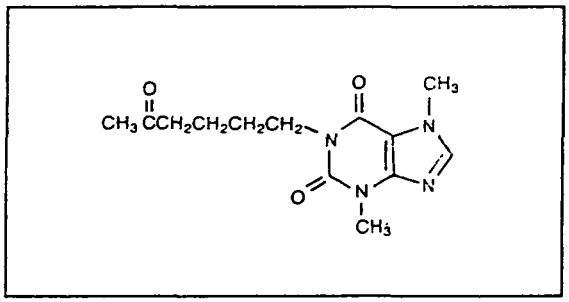

Figure 1. The structural formula of pentoxifylline.

laboratory animals has been assessed with LD_{50} doses, being in the order of 200 mg/kg when given i.v. When administered i.v. to pregnant rats at a dose of up to 12.5 mg/kg, there was normal prenatal development.

Action of pentoxifylline upon spermatozoa

The direct effects of the compound upon sperm function *in vitro* have been documented extensively, with brief exposure of the spermatozoa to pentoxifylline at concentrations of ~1 mg/ml usually resulting in the stimulation of various aspects of sperm motility (Rees *et al.*, 1990; Tesarik *et al.*, 1992b; Kay *et al.*, 1993; Lewis *et al.*, 1993). More importantly, pentoxifylline has been demonstrated to augment the response to factors which stimulate the acrosome reaction, such as follicular fluid and the ionophore A23187 (Tesarik *et al.*, 1992a; Carver-Ward *et al.*, 1994), as well as to improve the penetration of zona-free hamster oocytes (Hammitt *et al.*, 1989; Rees *et al.*, 1990; Lambert *et al.*, 1992) and binding to the zona pellucida in the hemizona assay (Yogev *et al.*, 1995). These facts support the notion that pentoxifylline can indeed stimulate the spermatozoa to undergo the acrosome reaction in response to a stimulus which mimics that of the vestments of the oocyte, and is useful in aiding the interaction of spermatozoa and oocytes within an assisted reproductive technology setting.

An alternative phosphodiesterase inhibitor, caffeine, has also been shown to stimulate sperm motility. However, it can stimulate the acrosome reaction itself, unlike pentoxifylline (Tesarik *et al.*, 1992a,b), and this premature shedding of the acrosomal cap may well be detrimental to the successful interaction of the spermatozoon with the zona pellucida. Tesarik *et al.* (1992a,b) also noted that pentoxifylline augmented the rate of acrosome reaction in response to follicular fluid and ionophore to a greater extent than did caffeine, confirming the greater potential for clinical utility.

Use of pentoxifylline to stimulate fertilization *in vitro*

The use of pentoxifylline was first described to improve fertilization *in vitro* at a time when the micromanipulation procedures were still in the early stages of development (Yovich *et al.*, 1987). The work using pentoxifylline had been prompted by a study presented at the Fertility Society of Australia the previous year comparing it with 2-deoxyadenosine (Yates *et al.*, 1986). The benefits seen within an IVF programme were first published by PIVET Medical Centre (Yovich *et al.*, 1988, 1990), and have been confirmed by others (Tesarik and Mendoza, 1993). However, the use of pentoxifylline to aid cases with failed (Tournaye *et al.*, 1993b) or reduced levels of fertilization (Tournaye *et al.*, 1994b) has been challenged, leading to a series of correspondence in the literature (Mills and Gadir, 1993; Tesarik, 1993; Tournaye *et al.*, 1993a). A closer examination of the reports do reveal methodological differences which may well be important in explaining the apparent discrepancy in the usefulness of pentoxifylline.

The first and most important point to note is that of patient selection. Following the initial reports of an overall benefit in relatively small select groups of men (Yovich *et al.*, 1988, 1990), but the recognition that pentoxifylline did not help everyone (Yovich *et al.*, 1990; Yovich, 1993), attention became focused on the identification of those individuals most likely to benefit. The PIVET group recognized that the augmentation of the acrosome reaction to ionophore challenge test by pentoxifylline was a good way of selecting appropriate cases (Yovich, 1993; Yovich *et al.*, 1994a), and it is interesting to note that Tesarik and Mendoza (1993) came to a similar conclusion in that those patients who showed the most benefit were those with a reduced rate of acrosome reaction induction in response to ionophore. The dissenting reports from Tournaye *et al.* (1993b, 1994b) did not attempt to select patients other than on the basis of a previous IVF treatment cycle. Indeed, the limitation of this blanket approach was answered by themselves in a later report (Tournaye *et al.*, 1994d), in which certain men with asthenozoospermia showed improvements in hyperactivation and induced acrosome reaction, leading to the conclusion that pentoxifylline 'may restore sperm func-

Table I. The use of intrauterine insemination to treat couples in which the man has a normal or reduced acrosome reaction to ionophore challenge (ARIC) test result (Yovich *et al.*, 1993)

ARIC result	Treatment	No. of cycles	Pregnancies
Normal	IUI only	161	24 (14.9)
Reduced	IUI only	71	2 (2.8)
Reduced	PTX + IUI	41	9 (22.0)

Values in parentheses are percentages. PTX = pentoxifylline treatment of spermatozoa; IUI = intrauterine insemination.

tion in certain of these patients, and perhaps improve fertilization *in vitro*, but in others it may produce no change or may even be detrimental to sperm function'.

Secondly, there are differences in the way in which the spermatozoa were treated with the pentoxifylline. Yovich *et al.* (1990) noted that a brief exposure of 30 min to pentoxifylline with immediate use of the treated spermatozoa gave the best clinical results in the IVF programme, compared with an extended culture of the treated and washed spermatozoa. The protocol adopted by Tournaye *et al.* (1993b) used an incubation time with the drug of 45 min. They then reduced this incubation to 30 min in their subsequent report (Tournaye *et al.*, 1994b), but oocytes were still only inseminated within 2 h of completing the sperm preparation. The precise significance of this extended culture of the washed spermatozoa is unclear, as many workers believe the effect of pentoxifylline after washing to persist for a few hours (Tesarik *et al.*, 1992b; Kay *et al.*, 1993), although oligozoospermic samples have been shown to exhibit an immediate improvement in motility characteristics which is lost within 1 h (Edirisinghe *et al.*, 1991). Once again, the key may lie in sample selection, with the poorer clinical samples being more susceptible to a detrimental effect of the pentoxifylline.

Use of pentoxifylline to stimulate fertilization *in vivo*

The oral administration of pentoxifylline by several workers has been reviewed (Tournaye *et al.*, 1994c). The initial rationale was to improve the blood flow to the testes (Heite, 1979), but the possible benefit of pentoxifylline upon the spermatozoa themselves was also considered (Aparicio *et al.*, 1980). There is conflicting evidence about the usefulness of pentoxifylline given in this manner. Coupled with the inevitable systemic effects seen with the administration of this drug, this approach is not popular.

Current work at the PIVET Medical Centre has concentrated on the treatment of spermatozoa *in vitro* for subsequent use by intrauterine insemination, whereby the oocytes can be fertilized within the female reproductive tract (Yovich *et al.*, 1993). Again, the selection of patients seems very important, with pentoxifylline being particularly beneficial in cases with a reduced acrosome reaction to ionophore challenge score as summarized in Table I. It should be noted that the inseminations were usually performed after follicular rupture, as confirmed by ultrasound. Therefore the treated spermatozoa would still have come into contact with the oocyte within a short time period after preparation.

Detrimental effects of pentoxifylline

The possible adverse effect of pentoxifylline on sperm function has already been suggested above. In essence, some clinical samples show a loss of motility characteristics after exposure to pentoxifylline (Tournaye *et al.*, 1994d), although this is not always observed (Tesarik *et al.*, 1992b). Nevertheless, the improvement in results obtained at PIVET with the protocol eliminating the post-preparation culture (Yovich *et al.*, 1990) must give credence to this possibility.

Pentoxifylline has a clear detrimental effect upon oocyte and embryo development (Tournaye *et al.*, 1993c,d; Scott and Smith, 1995), and so must be washed from the sperm preparations before the insemination of oocytes to avoid contamination of the IVF culture medium in which the human zygote will grow. This washing step has been said to be adequate (Yovich *et al.*, 1994b) and does not appear to be in dispute (Tournaye *et al.*, 1994a).

The place of pentoxifylline within an assisted reproductive technology programme

In summary, the pretreatment of spermatozoa with pentoxifylline should remain an option available in the treatment of male factor infertility. It is most effective in cases selected using tests of sperm function, such as the acrosome reaction to ionophore challenge test, rather than being applied wholesale. It can be used in both IVF and intrauterine insemination. Some patients may find attractive

and reassuring the use of an IVF technique in which the oocyte has some role in the selection of the fertilizing spermatozoon, and some clinics may appreciate the lower level of investment required to use the technique. In conclusion, the pretreatment of spermatozoa should be seen to complement the use of IVF and micromanipulation, giving clinics and patients greater choice.

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