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Toxicity of ornidazole and its analogues to rat spermatozoa as reflected in motility parameters

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Summary

Ornidazole, a 5-nitro-imidazole derivative, has contraceptive properties in rats. As some ornidazole passes through the body unmetabolized after administration, the aim of this study was to investigate if ornidazole itself has a direct effect on sperm motility and whether these effects are limited or potentiated by the epididymal epithelium or structural changes to the molecule. Cauda epididymal spermatozoa or cauda epididymal tubules were incubated with ornidazole or ornidazole analogues, and motility parameters were subsequently measured by means of a computer-assisted sperm analysis (CASA) system. Incubation of spermatozoa in 2.5 mmol/L ornidazole for 4 h reduced their motility significantly, whereas incubation of epididymal tubules for 8 h in 10 mmol/L ornidazole was required to alter the velocity parameters of the enclosed spermatozoa upon release, suggesting that extratubular non-metabolized ornidazole can participate in inhibiting the motility in vivo. The in vitro toxicity of ornidazole derivatives depends on the halogen present and on the position of the nitro-group. The putatively inactive (R)- and the active (S)-ornidazole exhibited equivalent depression of sperm motility by direct incubation. This observation, and the differences between the in vitro and the in vivo efficacies of various ornidazole analogues, indicates distinct mechanisms of motility inhibition in the two experimental systems.

Keywords: ornidazole, rat, sperm motility, spermatozoa, toxicity

Introduction

The antimicrobial agent ornidazole [1-(3-chloro-2-hydroxy)propyl-2-methyl-5-nitroimidazole] has antifertility action in male rats (McClain & Downing, 1988a, b; Oberländer *et al.*, 1994). Its advantages as an antifertility agent are the rapid onset of infertility after oral gavage, the fast recovery after its withdrawal and the minor side effects caused. The first two observations indicate that the drug acts on spermatozoa while they are in the epididymis. Spermatozoa released from the cauda epididymis of ornidazole-treated rats into a glucose-containing medium free of lactate and pyruvate display decreased motility parameters (Toth *et al.*, 1992; Yeung *et al.*, 1995), similar to that found in α-chlorohydrin treated rats (Toth *et al.*, 1992). This effect is mediated by energy deprivation of the spermatozoa due to inhibition of the glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase and triose phosphate isomerase in epididymal spermatozoa (Oberländer *et al.*, 1996).

Recently, the fertility of male rats fed nitroimidazole derivatives, differing from ornidazole only in the carbon side chain, was studied (Cooper *et al.*, 1997). These compounds were given by oral gavage in equimolar amounts to the antifertility dose of ornidazole, and only ornidazole and its acetate exhibited antifertility effects as shown by a reduction in the number of embryos per corpus luteum. Other analogous chemical structures lacking the Cl-atom or the propyl-side chain displayed no antifertility effects, suggesting

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that the entire ornidazole molecule or the three-carbon side chain were the effective agents.

During metabolism in the rat, ornidazole is partly cleaved of its 3-chloro side chain (Schwartz *et al*., 1979), and Jones & Cooper (1997) demonstrated the appearance in urine of 3-chlorolactate, an oxidation product of 3-chlorolactaldehyde. The authors suggested that ornidazole exerts its antifertility effect via the liberation of 3-chlorolactaldehyde, because (S)-3-chlorolactaldehyde is an inhibitor of glyceraldehyde-3-phosphate-dehydrogenase in boar spermatozoa (Jones & Stevenson, 1983; Stevenson & Jones, 1985). (S)-α-chlorohydrin, 6-chloro-6-deoxy D-glucose and other 6-chloro-6-deoxy D-sugars are other putative (S)-3-chlorolactaldehyde precursors and all are effective male contraceptives in the rat. By contrast, the optical isomer (R)-α-chlorohydrin does not exhibit antifertility properties (Jackson & Robinson, 1976; Jackson *et al*., 1977), but causes renal toxicity in rats (Porter & Jones, 1982; Stevenson & Jones, 1984).

Jones & Cooper (1997) demonstrated that within an interval of 7 h after oral gavage of ^{36}Cl-ornidazole to rats, 25% of the radioactivity administered was recovered unchanged in urine. In addition, Schwartz *et al*. (1979) reported that at least 15% of the administered ^{14}C-ornidazole remained unchanged in the urine of rats over the 2 days after administration. Therefore, it could be that ornidazole itself can enter the epididymal lumen and have a direct effect on spermatozoa, and that conversion to an active metabolite in the body is not necessary.

The aim of the present study was to establish (1) whether ornidazole has a direct effect on the motility of spermatozoa, (2) whether the epithelium of the epididymal tubule reduces or enhances the effect of ornidazole on sperm motility, and (3) whether a change in the nitroimidazole structure (changes in the halogen, the position of the nitro-group, (R)- and (S)-enantiomers) has any effect on sperm motility. Spermatozoa and tubules from rat cauda epididymides were isolated and incubated in a glucose-containing medium without lactate and pyruvate, which contained different ornidazole analogues at various concentrations. After incubation, kinematic parameters were analysed with a computer aided sperm analysis (CASA) system.

Materials and methods

Chemicals

Ornidazole and the ornidazole analogues were obtained from different sources, as listed in Table 1. The synthesized compounds were characterized by nuclear resonance spectroscopy (NMR) (Skupin *et al*., 1997). Other chemicals were purchased from Sigma Chemie (Deisenhofen, Germany) unless otherwise stated.

Animals

Adult male Sprague Dawley rats (350–550 g) were purchased from Charles River Wiga GmbH, Sulzfeld, Germany. Experiments were undertaken in accordance with guidelines of the German code for animal experiments.

Isolation and incubation of cauda epididymal spermatozoa

Rats were anaesthetized by intraperitoneal injection of 0.5 mL 30% urethane (ethyl carbamate) per 100 g body weight. One testis with its attached epididymis was excised and blotted. The epididymis was then dissected from the testis and the cauda region separated. After removal of the adjacent fat pad the blunt tip of a 26G needle shaft connected to a syringe via PVC tubing (Dural Plastics, Australia, 0.5 mm i.d., 0.8 mm o.d.) was tied into the ductus deferens.

Table 1. Names of the compounds used in this study and their source

Code in Figs 5 and 6	Common name	Chemical name	Source
A	–	2-methyl-5-nitroimidazole	Acros Chimica, Geel
B	dimetridazole	1,2-dimethyl-5-nitroimidazole	Sigma, Deisenhofen
C	metronidazole	1-(2-hydroxy)ethyl-2-methyl-5-nitroimidazole	Sigma, Deisenhofen
D	–	1(2-chloroethyl) 2-methyl 5-nitroimidazole	Aldrich, Steinheim
E	tinidazole	1-(2-sulfonylethyl)ethyl-2-methyl-5-nitroimidazole	Sigma, Deisenhofen
F	ornidazole epoxide	1-(2,3-epoxy)propyl-2-methyl-5-nitroimidazole	synthesized
G	ornidazole acetate	1-(3-chloro-2-acetoxy)propyl-2-methyl-5-nitroimidazole	synthesized
V	fluoro-ornidazole	1-(3-fluoro-2-hydroxy)propyl-2-methyl-5-nitroimidazole	synthesized
W	4-nitro-fluoro-ornidazole	1-(3-fluoro-2-hydroxy)propyl-2-methyl-4-nitroimidazole	synthesized
X	(R,S)-ornidazole	(R,S)-1-(3-chloro-2-hydroxy)propyl-2-methyl-5-nitroimidazole	Sigma, Deisenhofen
–	(R)-ornidazole	(R)-1-(3-chloro-2-hydroxy)propyl-2-methyl-5-nitroimidazole	synthesized
–	(S)-ornidazole	(S)-1-(3-chloro-2-hydroxy)propyl-2-methyl-5-nitroimidazole	synthesized
Y	4-nitro-ornidazole	1-(3-chloro-2-hydroxy)propyl-2-methyl-4-nitroimidazole	synthesized
Z	bromo-ornidazole	1-(3-bromo-2-hydroxy)propyl-2-methyl-5-nitroimidazole	synthesized

After cutting the epididymal tubule exposed at the caudal flexure, the epididymal contents were flushed from the tubule by introduction of PBS (phosphate buffered saline, pH 7.4, Gibco, Life Technologies) from the syringe.

The recovered luminal contents were collected with a positive displacement pipette (Microman, Gilson) and 3 μL was released in 27 μL medium G containing 135 mmol/L NaCl, 4 mmol/L KCl, 2 mmol/L $CaCl_2$, 0.4 mmol/L $MgSO_4$, 0.3 mmol/L Na_2HPO_4, 8 μg/mL phenol red, 25 mmol/L $NaHCO_3$, 5 mmol/L glucose and 4 mg/mL bovine serum albumin (pH 7.4) and the specified concentration of the tested drug. The suspensions were incubated for 4 h at 34 °C (the temperature of the cauda epididymis) in 5% CO_2. More sperm suspensions were prepared later from the cauda epididymis on the other side of the anaesthetized rat as described above.

Isolation and incubation of cauda epididymal tubules

The rats were first anaesthetized as above and the testis with the attached epididymis excised. The epididymal tail was placed in a Petri dish containing medium 199 (M 199, without lactate and pyruvate) and after removing the capsule, the connective tissue was dissected with jeweller's forceps. After gently unravelling the tubule, it was double-ligated every centimetre and cut between the ligatures into segments. These ligated segments were then incubated at 34 °C in 5% CO_2 in medium 199 containing different concentrations of ornidazole for 8 h. At the end of incubation spermatozoa from the tubule segments were released into 10 μL medium G, placed on a small plastic spatula and further diluted for the recording.

Analysis of sperm motility

At the end of incubation, the spermatozoa were diluted about 300 times with medium G, and a volume of 13 μL was placed on a heated (35 °C) and siliconized slide and coverslipped (18 × 18 mm). A 4 min video recording of the spermatozoa from different fields on the heated slide was made using a phase contrast microscope (Olympus BH-2 at magnifications: objective 4× and photo-ocular 3.3×, pseudo-darkfield: phase ring 40). The percentage motility (MOT) was estimated manually by counting at least 200 flagellating or non-flagellating spermatozoa. At least 100 sperm tracks from the recording were analysed by CASA (Hamilton-Thorne, Beverly, Massachusetts, HTM-MASTER C-system, version 10.6 h). Motility parameters shown in this paper comprise average path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL), straightness (STR = VSL/VAP × 100) and linearity (LIN = VSL /VCL × 100). The following settings were used: frame rate: 12.5 Hz, number of frames analysed: 30, minimum contrast: 30, minimum size: 20, magnification: 0.63, illumination intensity: 2150, minimum number of detected data points of a track: 10, minimum VAP: 10 μm/sec, minimum VCL: 15 μm/sec.

Statistics

For each sample, kinematic parameters were expressed as the median of at least 100 analysed sperm tracks per animal. The data mentioned in the text and graphs are the mean values of each treatment group. All values expressed in percentage were transformed by arc-sin square-root conversion for statistical evaluation and the retransformed mean values were used for the expression of these data. The standard error of the mean (SEM) is given as the difference of the retransformed mean + SEM and the retransformed mean values. Differences in motility and kinematic parameters between different times of incubation, types and concentrations of drugs were calculated by one-way analysis of variance (95% LSD, Student-Newman-Keuls-Method). The calculations were undertaken using means of the Statgraphics (Version 5, STSC, Inc., Rockville, Maryland) or the SigmaStat-program (Version 1.0, Jandel Corporation).

Results

Effect of basal incubation conditions on sperm motility parameters

An incubation time of 6 h had no significant effect on VAP, VSL and STR of non-treated spermatozoa, whereas a slight but significant decrease in VCL was observed by 4 h (Fig. 1). However, there was no further decrease of VCL after 6 h. LIN increased, though not significantly, from 56.7 (±9.9)% after 2 h to 72.3 (±8.7)% (mean ± SEM). Only the percentage motility decreased continuously with time (61.8 ± 10.4% at 2 h to 21.3 ± 5.1% at 6 h). On account of the decreasing percentage motility, the 4 h incubation was chosen for subsequent studies. For monitoring the motility of spermatozoa incubated within epididymal tubules, an incubation interval of 8 h was preferred, because a shorter incubation time in ornidazole revealed no, or little, effect of the drug (data not shown).

Comparison of direct and indirect incubation of spermatozoa in (R,S)-ornidazole

Direct incubation of spermatozoa for 4 h in ornidazole had a more powerful effect on sperm motility than did the incubation of spermatozoa within an epididymal tubule for 8 h (Fig. 2). Spermatozoa incubated in medium G containing 2.5 mmol/L ornidazole displayed a reduction in VSL ($p < 0.05$), VCL and LIN, although the percentage motility was not decreased by this concentration. Concentrations of 5 mmol/L and 10 mmol/L ornidazole, however, led to a near complete cessation of motility, and subsequently all other kinematic parameters. In contrast, incubation of the tubules with 20 mmol/L ornidazole had no significant effect ($p > 0.05$) on VCL and the percentage motility even after incubation for 8 h. The VSL and the LIN of spermatozoa incubated within their epididymal tubule decreased with an ornidazole concentration of 10 mmol/L (52.7% of VSL-

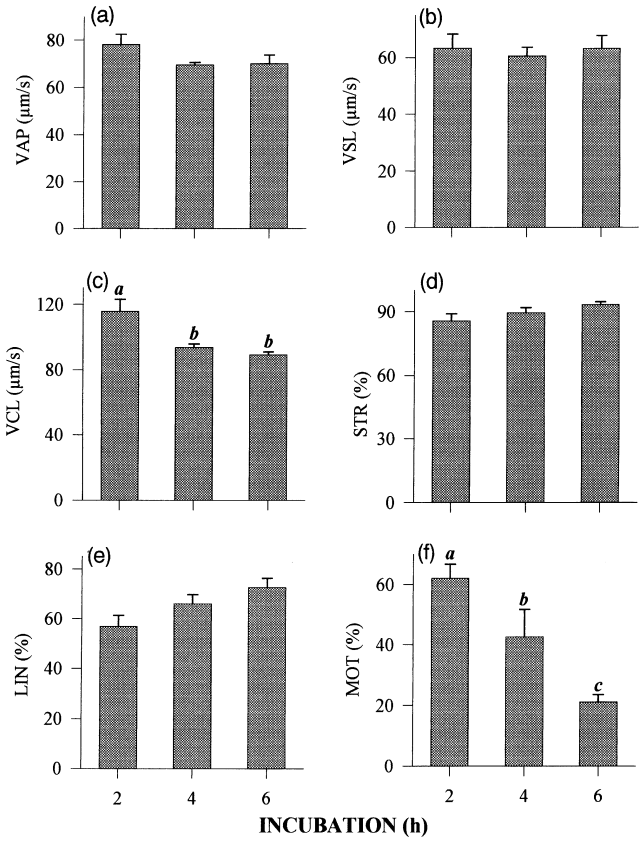

Figure 1. Motility parameters [a, VAP; b, VSL; c, VCL; d, STR; e, LIN, f, MOT (%)] of rat cauda epididymal spermatozoa incubated in medium G for 2, 4 or 6 h. Values are means + SEM ($n = 6$). Values with different letters are statistically significantly different ($p < 0.05$).

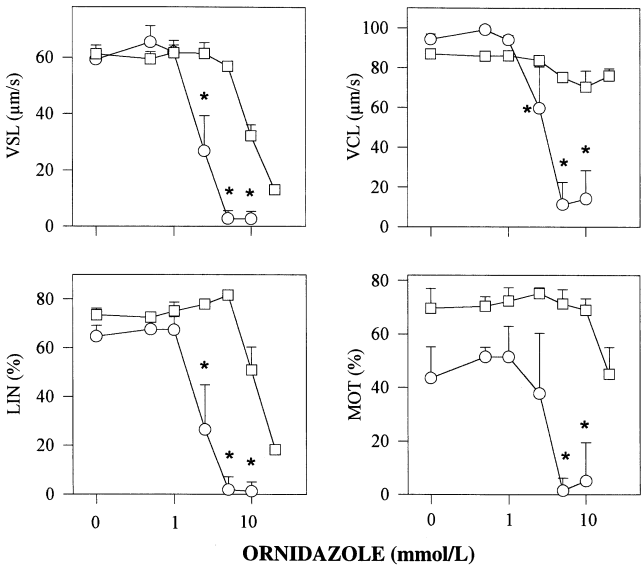

Figure 2. Motility parameters of cauda epididymal spermatozoa incubated directly (circles) for 4 h or within epididymal tubules (squares) for 8 h in medium G containing different concentrations of ornidazole. Values are means + SEM ($n = 4$, except 20 mmol/L in tubule incubation: $n = 3$). Values with asterisks indicate significant differences ($p < 0.05$) between the two procedures at the same drug concentration.

control and 69.7% of LIN-control) and 20 mmol/L (21.2% of VSL-control and 25.0% of LIN-control).

Effect of enantiomers of ornidazoles

A comparison of the motility-inhibiting efficacy of (R)- and (S)-ornidazole (Fig. 3) on spermatozoa directly incubated for 4 h revealed no differences between the enantiomers (Fig. 4). VSL, VCL and LIN decreased at concentrations of between 1 and 5 mmol/L of the racemic mixture of ornidazole as well as the enantiomers separately.

Screening of ornidazole analogues with a different side chain at N (position 1)

Spermatozoa incubated directly for 4 h in 5 mmol/L ornidazole, dimetridazole, 1-(2-chloroethyl)-2-methyl-5-nitroimidazole, ornidazole epoxide, bromo-ornidazole and fluoro-ornidazole exhibited significantly decreased kinematic parameters, whereas metronidazole, tinidazole, 2-methyl-5-

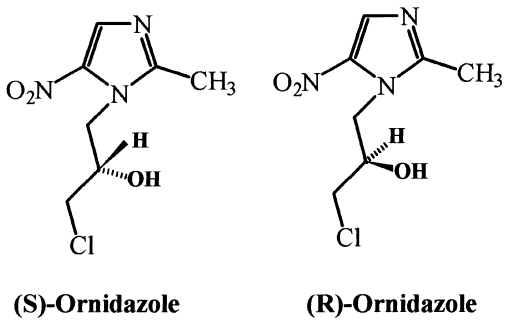

Figure 3. Stereochemistry of the enantiomers of ornidazole.

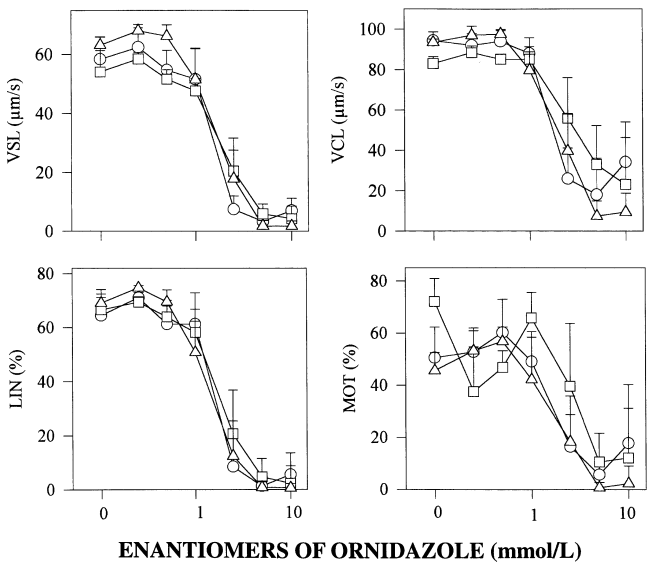

Figure 4. Motility parameters of cauda epididymal spermatozoa incubated directly in medium G containing different concentrations of (R)- (○) (S)- (□) or (R,S) (△) ornidazole. Values are means + SEM. [$n = 4$ for (R)- and (S)-ornidazole and $n = 6$ for (R,S)-ornidazole.]

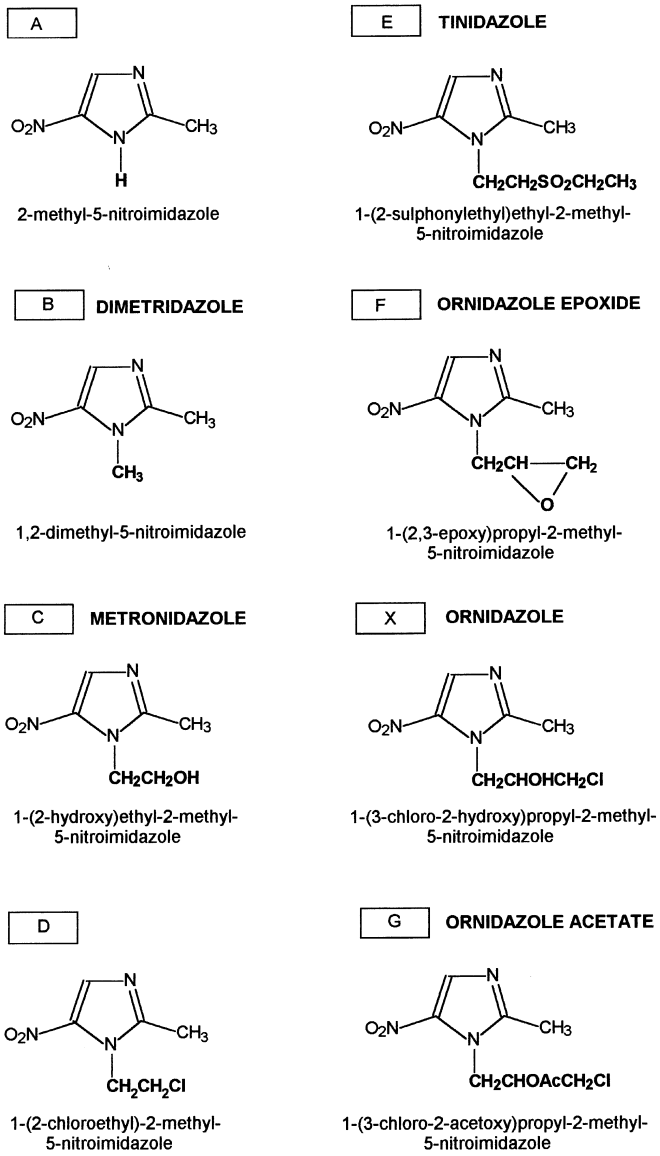

Figure 5. Structures of ornidazole analogues differing in the carbon side chain.

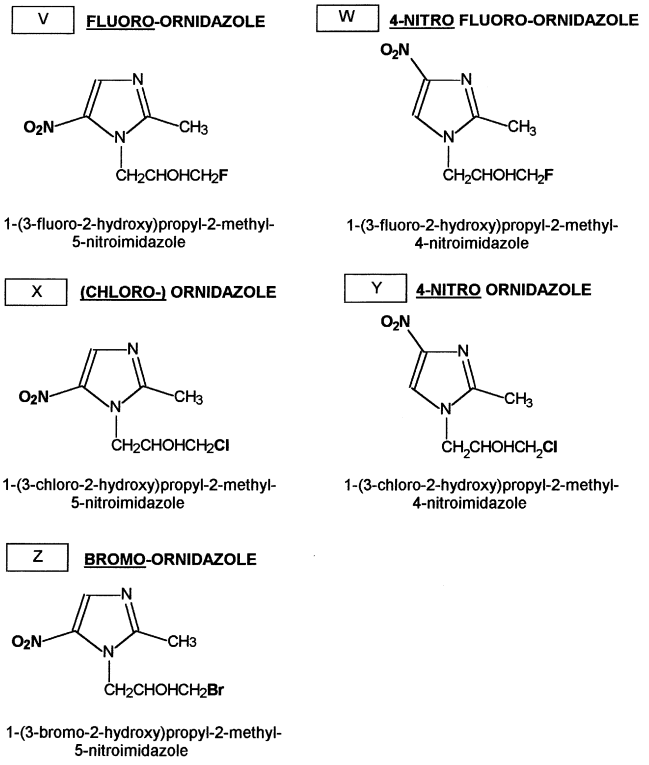

Figure 6. Structures of ornidazole analogues differing in the halogen on the carbon side chain and/or in the position of the nitro-group.

nitroimidazole, 4-nitro-ornidazole and 4-nitro-fluoro-ornidazole (Figs 5 & 6) had no significant effects (Fig. 7). All of the estimated velocities of spermatozoa were decreased significantly when incubated in 5 mmol/L ornidazole-acetate, whereas no significant decline in STR, LIN and MOT was demonstrable.

Screening of ornidazole analogues with the nitro-group in different positions

Another group of compounds tested differed only in the position of the nitro-group attached to the imidazole ring of ornidazole and fluoro-ornidazole (Fig. 6). The compounds with the 4-nitro-group exhibited no, or only a small, inhibitory effect on the motility of spermatozoa after 4 h incubation, whereas the 5-nitro-compounds decreased the motility at comparable concentrations (2.5 up to 10 mmol/L in ornidazole and 5–20 mmol/L in fluoro-ornidazole) irrespective of the nature of the halogen substituent (Figs 8 & 9).

Screening of ornidazole analogues with different halogens

These compounds differed only in the halogen on the three carbon side chain (Fig. 6). Bromo-ornidazole was the most effective at inhibiting sperm motility, followed by chloro- and fluoro-ornidazole (Fig. 10). These different grades of motility efficacy were demonstrated for all motility parameters.

Discussion

In this study, a medium containing glucose as substrate without pyruvate and lactate was used to reveal anti-motility effects caused by a putative energy deprivation mechanism as a result of reduced glycolysis in spermatozoa. Yeung *et al.* (1995) showed that motility parameters of spermatozoa obtained from rats fed 400 mg ornidazole/day/kg body-weight were most markedly reduced when glucose was the only substrate in the incubation medium. This decrease in motility reflected the reduced activities of the glycolytic enzymes glyceraldehyde 3-phosphate dehydrogenase and triose phosphate isomerase in the spermatozoa (Oberländer *et al.*, 1996). The incubation conditions selected here supported the motility of rat cauda epididymal spermatozoa,

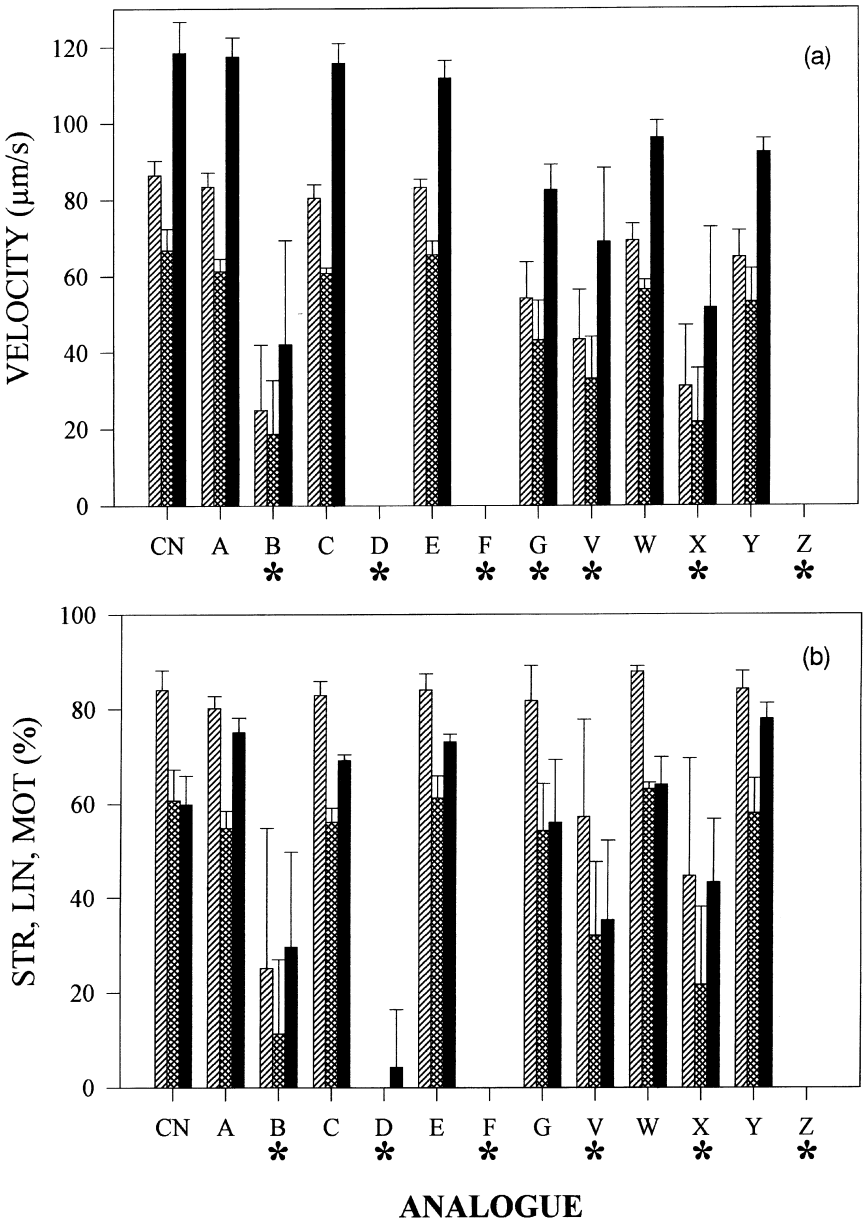

Figure 7. Motility parameters (a, velocity: VAP (hatched bars), VSL (cross-harched bars), VCL (solid bars); b, derived parameters: STR (hatched bars), LIN (cross-hatched bars), MOT (solid bars) of cauda epididymal spermatozoa incubated directly in medium G containing different drugs at 5 mmol/L [CN, control; A, 2-methyl-5-nitro-imidazole; B, dimetridazole. C, metronidazole; D, 1-(2-chloroethyl) 2-methyl-5-nitro-imidazole; E, tinidazole; F, ornidazole epoxide; G, ornidazole acetate; V, fluoro-ornidazole; W, 4-nitro-fluoro-ornidazole; X, ornidazole; Y, 4-nitro-ornidazole; Z, bromo-ornidazole]. Values are means + SEM ($n = 4$). Columns with asterisks are statistically significantly different ($p < 0.05$) from the control for all parameters.

and only the percentage motility was reduced after 6 h incubation. Therefore an incubation time of 4 h was chosen for further studies. It should be noted that it was easier to maintain the potential for motility of spermatozoa retained undiluted within their epididymal tubule.

Under the direct incubation conditions used, ornidazole inhibited sperm motility at a concentration of 2.5 mmol/L. This suggests that non-metabolized ornidazole circulating in the blood of the ornidazole-fed rats (Schwartz *et al.*, 1979; Jones & Cooper, 1997) and man (Taburet *et al.*, 1989) could contribute to the antifertility effect.

A prerequisite for a direct effect of ornidazole on spermatozoa in vivo is the presence of ornidazole in the lumen of the epididymis or the conversion by the epididymis into an active metabolite of ornidazole and its transport into the lumen. Thus, in these experiments, epididymal tubules with their contained spermatozoa were incubated in ornidazole. Indeed, the motility of spermatozoa subsequently released from these tubules was affected only after 8 h incubation in 10 mmol/L ornidazole, suggesting that the epithelium of the epididymal tubule is an incomplete barrier or may even participate in the conversion of ornidazole into an inhibitory compound. The demonstrated reduction in LIN and VSL, and the maintained VCL and percentage motility of spermatozoa in the tubule incubations, differed markedly from the effects on spermatozoa incubated directly in drugs, when all parameters decreased simultaneously. This indicates that the direct action of ornidazole on spermatozoa is different from that on spermatozoa in the tubule and suggests that the epithelium of the tubule mediates the

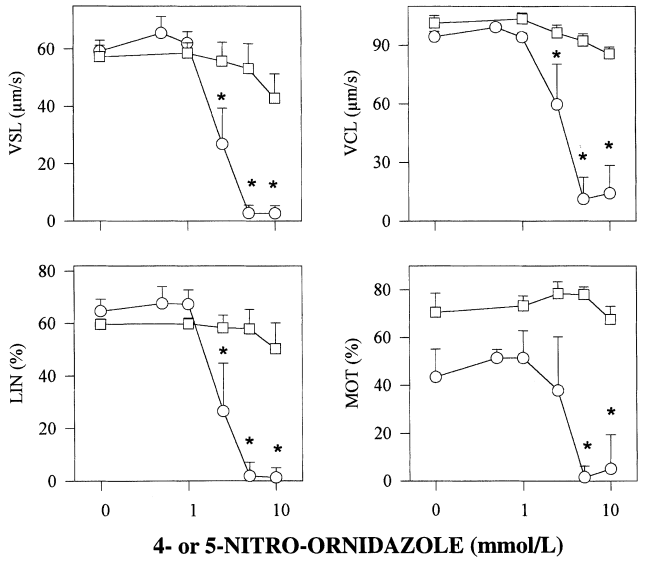

Figure 8. Motility parameters (ordinate) of cauda epididymal sperm incubated directly in medium G containing different concentrations of 4-nitro-ornidazole (□) and (5-nitro-)ornidazole (○) (abscissa). Values are mean + SEM ($n = 4$). Values with asterisks indicate significant differences between the two isomers at the same concentration.

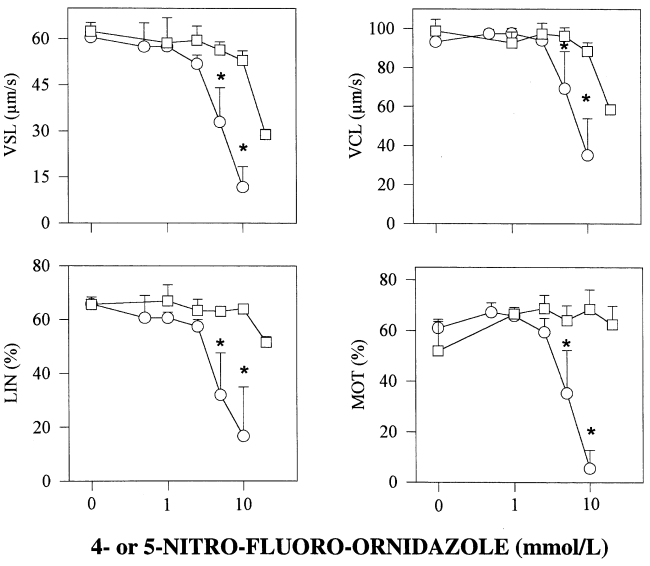

Figure 9. Motility parameters of cauda epididymal spermatozoa incubated directly in medium G containing different concentrations of 4-nitro-fluoro-ornidazole (□, $n = 4$, except 0.5 mmol/L: $n = 2$) and 5-nitro-fluoro-ornidazole (○, $n = 5$, except 0.1 mmol/L: $n = 2$). Values are means + SEM. Values with asterisks indicate significant differences ($p < 0.05$) between the two isomers at the same concentration.

conversion of ornidazole, or limits the amount of ornidazole entering the lumen. In conclusion, these results support the view that extratubular unconverted ornidazole could be responsible for the decline in sperm motility in addition to metabolites produced in the body of the animal in vivo.

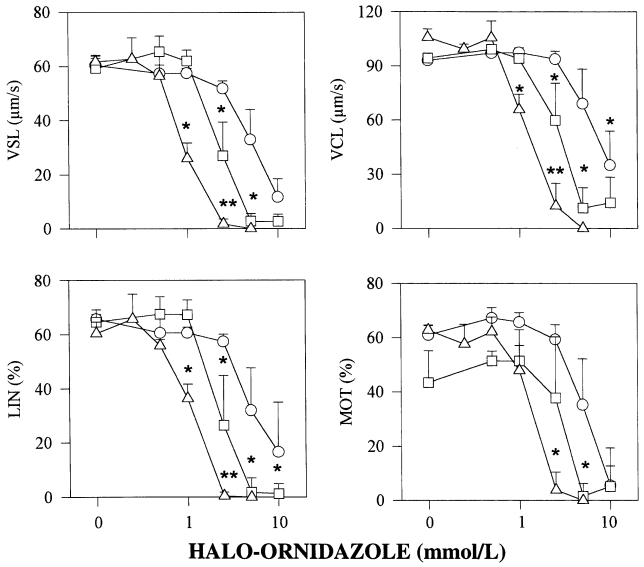

Figure 10. Motility parameters of cauda epididymal spermatozoa incubated directly in medium G containing different concentrations of fluoro-ornidazole (○), (chloro)-ornidazole (□) and bromo-ornidazole (△). Values are means + SEM ($n = 4$, except 0.5 mmol/L: $n = 2$). Values with one or two asterisks indicate statistically significantly differences ($p < 0.05$) between the three halogenated derivatives at the same concentration.

A comparison of the actions of nitroimidazole analogues also demonstrates differences between the motility inhibiting efficacy when the drugs were given by oral gavage (Cooper *et al.*, 1997) and the effects seen here on motility in vitro. Cooper *et al.* (1997) administered 1.8 mmol/kg/day of ornidazole analogues to rats. Only ornidazole, its acetate and bromo-ornidazole inhibited motility and the former two exerted an antifertility effect as well, as shown by a reduction in the number of embryos per corpus luteum, whereas dimetridazole, metronidazole, 1-(2-chloroethyl)-2-methyl-5-nitro imidazole, tinidazole, 2-methyl-5-nitroimidazole and ornidazole epoxide were without such an effect. Comparison with the motility inhibiting efficacy of these compounds at 5 mmol/L in vitro, revealed differences concerning dimetridazole, 1-(2-chloroethyl)-2-methyl-5-nitroimidazole and ornidazole epoxide compared with in vivo experiments which showed no inhibition of motility. The differences between the in vivo and in vitro efficacy of these compounds indicate distinct mechanisms of action, and thus the in vitro method cannot be used as a screening model for antifertility and motility inhibiting effects in vivo.

Separate mechanisms of in vivo and in vitro action were further indicated by the incubation of spermatozoa with the enantiomers (R)- and (S)-ornidazole. In vivo, ornidazole is at least partly cleaved of its three carbon chloro-side chain (Schwartz *et al.*, 1979; Jones & Cooper, 1997), which is probably converted into 3-chlorolactaldehyde, the active inhibitor of glyceraldehyde 3-phosphate dehydrogenase (Jones & Stevenson, 1983; Stevenson & Jones, 1985). It has been shown (Stevenson & Jones, 1982, 1984) that only

the (S)-enantiomer of alpha-chlorohydrin or 3-chlorolactaldehyde have the capability of inhibiting glycolysis of spermatozoa. If ornidazole were converted into 3-chlorolactaldehyde during incubation, a different efficacy of (R)- and (S)-ornidazole would be anticipated, but the actions of both enantiomers in vitro were identical. Thus it is concluded that ornidazole effects the motility of spermatozoa in vitro as the entire molecule.

There are a few possible reported mechanisms as to how 5-nitro-imidazoles might act directly. Rao *et al.* (1988) reported that intact rat hepatocytes generate nitro-furantoin radical anions and other nitro anion radicals from ornidazole. This may be an additional factor responsible for the different effects in vivo and in vitro, because spermatozoa may produce more of these radicals in vitro than in vivo, owing to a more direct effect of ornidazole and the absence of free radical protection systems.

It is also known that 5-nitro-imidazoles act by irreversibly accepting electrons from reduced ferredoxin in clostridiads (Edwards *et al.*, 1973). Owing to this electron-scavanging process the H_2 evolution of the phosphoroclastic system is inhibited and therefore clostridium cannot produce enough energy to survive. Although the oxidative phosphorylation system in spermatozoa is different, it does employ an electron-acceptor-system and it could be that ornidazole has a similar inhibitory effect in vitro.

To highlight the role of the position of the nitro group attached to the imidazole ring, the effects of 4-nitro and 5-nitro-imidazole derivatives were investigated. The compounds containing the 4-nitro-groups (both chloro- and fluoro-ornidazole) displayed no, or only a small, effect on the motility of spermatozoa, whereas the 5-nitro-compounds were potent inhibitors at comparable concentrations. Interestingly, Cosar *et al.* (1966) reported that 5-nitro-imidazole had a greater effect on the death of trichonomads than did 4-nitro-imidazole.

In an additional set of experiments, the chlorine at the three carbon side chain was exchanged for fluorine or bromine. These halogens possess considerable differences in their electronegativity (after Pauling: Br: 2.8, Cl: 3.0, F: 4.0: Latscha & Klein, 1996) causing different distributions of charge in the whole nitro-imidazole derivative. The bromo-derivative had the highest inhibitory effect on sperm motility, followed by the chloro- and the fluoro- derivative. These results suggest that the toxicity of 1-(3-halo-2-hydroxy)propyl-2-methyl-5-nitroimidazoles to spermatozoa is inversely proportional to the electronegativity of the halogen. The bromo-compound, administered by oral gavage to rats at a dose of 1.82 mmol/kg/day, caused paresis of the hind limbs after 10 days (Cooper *et al.*, 1997), which demonstrates the higher toxicity of this compound as found in vitro.

In conclusion, the in vitro incubation of rat spermatozoa with nitroimidazole drugs, followed by objective analysis of motility parameters, does not reflect the effect of these compounds on fertility or motility when given orally to rats. The inhibition of motility caused by ornidazole is mediated by different mechanisms in vivo and in vitro. The epididymal tubule acts as an incomplete barrier for unmetabolized extratubular ornidazole, but incubation of spermatozoa within the epididymal tubule evokes a change in motility parameter patterns different from direct incubations. The effects of different nitro-imidazole analogues in vitro reflect the toxicity of these compounds to spermatozoa as well as their general toxicity.

Acknowledgements

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