EFFECTS OF ENALAPRIL ON HUMAN SPERM MOTILITY

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ABSTRACT

The angiotensin converting enzyme inhibitor, enalapril, reduced the motility of washed human spermatozoa. The concentrations producing 50% inhibition were: (percentage motility) 11.6 ± 2.6 mM, (average forward velocity) 8.0 ± 1.5 m/s and (motility index) 0.5 ± 2.1 (mean ± s.e.m.). Enalapril 20 mM prevented all forward movement and reduced percentage motility to an extremely low level. Motility was reduced within 20 sec of addition and little further change occurred during a 60-min incubation period. Inhibition of percentage motility by enalapril was reversible with a 5-min washing procedure following a 5-min incubation period, but not following a 60-min incubation period. Enalapril 20 mM did not impair plasma membrane integrity (hypoosmotic swelling test) or viability (nigrosin-eosin stain). It is concluded that the antimotility effects of enalapril are not caused by inhibition of angiotensin converting enzyme, but may be of interest in the search for new spermicidal agents.

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INTRODUCTION

Several studies have shown the presence of angiotensin converting enzyme (ACE) in the male reproductive tract including the testes, epididymis, vas deferens and prostate (1,2,3). Recently ACE has also been shown to be present in spermatozoa (4,5) and in semen (6). However, the precise role of ACE in male reproductive function is unknown.

The ACE inhibitor captopril impairs motility of human spermatozoa (7). Moreover, the clinically effective male antifertility agent gossypol reduces sperm motility and inhibits ACE (8). It is possible that ACE may play a role in the regulation of sperm motility and also that captopril or a related compound may be worth evaluating as a potential contraceptive agent.

This paper reports the antimotility effect, on human spermatozoa, of enalapril, which is the most potent ACE inhibitor in clinical use (9).

METHODS

A single semen sample was obtained from each of 9 healthy donors (25–30 years) following 3–5 days of sexual abstinence. The specimens were allowed to liquefy at 31°C for 15–25 min, and then standard semen analysis was undertaken under phase contrast microscopy according to the guidelines established by the World Health Organization (10). All specimens used had a sperm concentration \( \geq 50 \times 10^6 \) spermatozoa/ml, motility of \( \geq 40\% \) and normal morphology of \( \geq 40\% \).

The spermatozoa were isolated from seminal plasma by three cycles of centrifugation (500 g for 5 min) with 9 ml volumes of medium BWW (11) and were finally resuspended at a density of \( 20 \times 10^6 \) spermatozoa/ml for the evaluation of drug effects. Enalapril was dissolved in BWW medium, 500 μl of washed spermatozoa suspension was placed in Falcon tubes and an equal volume of either the vehicle (BWW) or enalapril dissolved in BWW was added (final concentration of enalapril 2.5, 5.0, 10.0 or 20.0 mM), mixed well and the time was recorded. The suspensions were incubated for 60 min and 10 μl samples were transferred onto separate clean glass slides (31°C) and covered with a cover glass (22 x 22 mm). The percentage motile spermatozoa (10) and the average forward velocity (12) were determined at each concentration and time point under phase contrast microscopy using a squared grid and a crossed micrometer, respectively. The motility index was then calculated as the product of percentage motility and velocity (13). The spermatozoa incubated with 20 mM enalapril for 5 min and 60 min were washed with BWW for 5 min and the above mentioned motility parameters were reinvestigated. Further samples of enalapril-treated (20 mM) and control spermatozoa were subjected to the hypoosmotic swelling test (14) and to the nigrosin-eosin stain technique at 5 min and 60 min, respectively. The concentration of enalapril that decreased the percentage spermatozoa motility, the average forward velocity and the motility index to 50% of control (EC50) was obtained from log concentration response curves.

Results are given as means ± s.e.m. Statistical comparisons were made using Student's Paired t-test.
CONTRACEPTION

RESULTS

Enalapril inhibited the motility of human spermatozoa. At a concentration of 10 mM, enalapril inhibited average forward velocity, percentage motility and motility index (Figure). Motility was found to be inhibited immediately after addition of enalapril and there was little change in the level of inhibition during the subsequent 60 min. At the higher concentration of 20 mM, enalapril caused complete abolition of forward velocity and motility index, and the percentage motility was extremely low (Figure). In none of the suspensions treated with enalapril was there any flocculation or agglutination. The EC₅₀ values for enalapril were: (inhibition of percentage motility) 11.6 ± 2.6 mM, (average forward velocity) 8.0 ± 1.5 mM and (motility index) 8.5 ± 2.1 mM.

The effects of enalapril on forward velocity and on motility index appeared not to be reversible on washing. However, the percentage motility was restored by a 5-min wash to 67±0.1% of control motility (n=9, P<0.05) when prior incubation with enalapril had been 5 min. When enalapril had been in contact with the spermatozoa for 60 min, a 5-min wash did not restore motility (7.5±9.3% of control percentage motility, n=9, not significantly different from enalapril-treated suspensions).

Enalapril 20 mM had no effect on viability (nigrosin-eosin stain technique) or hypoosmotic swelling, after either 5 or 60 min contact. At 60 min the viability of control suspensions was 66±4%, and of enalapril 20 mM suspensions was 68±5%. At 60 min the control samples contained 61±6% swollen spermatozoa and the enalapril 20 mM suspension contained 53±3% swollen spermatozoa (n=9, not significantly different).

DISCUSSION

This study demonstrates a concentration-related antimotility effect of the ACE inhibitor enalapril in washed human spermatozoa in vitro. However, the potency ratio of the ACE inhibitors captopril and enalapril for reduction of motility of human spermatozoa is about 0.5 (7) and for inhibitors of ACE about 0.1 (15). Thus, the sperm immobilization effect of enalapril is not likely to be the result of inhibition of ACE, which has been shown to be present on spermatozoa (4,5). The ACE inhibitors block both forms of ACE, and also inhibit other dipeptidyl carboxypeptidases (16). Other actions of enalapril that could mediate its antimotility effect include reducing cyclic AMP concentration (17), reducing bradykinin levels (18), or increasing prostaglandin synthesis (19). These effects would be expected to have a deleterious effect on sperm function (20). Several drugs immobilize spermatozoa through stabilization of the cell membrane (21). Since enalapril is hydrophilic (22), it is unlikely to stabilize the sperm membrane. Placzek et al. (8) reported that neither enalapril nor captopril affects sperm motility, but in that study the concentrations used were too low. Our results show that when the concentration of enalapril or captopril is increased slightly above the maximum used by Placzek et al., a clear antimotility effect is seen.

Of the semen parameters measured, enalapril is more specific than captopril as an inhibitor of sperm motility. At concentrations producing complete abolition of forward velocity, enalapril did not impair membrane function or...
Figure.

Inhibition by enalapril of (a) forward velocity, (b) percentage motility and (c) motility index of washed human spermatozoa. x = vehicle control, ▲ = enalapril 2.5 mM, ■ = enalapril 5 mM, ○ = enalapril 10 mM, ● = enalapril 20 mM. Mean ± s.e.m., n = 9 donors.

* = P<0.05, ** = P<0.01, *** = P<0.001 compared with vehicle control.
viability as judged by the hypoosmotic swelling test and the nigrosin-eosin stain test. By contrast, captopril impairs membrane function in parallel with loss of movement (7).

A close relationship exists between sperm movement and fertility (23). Ejaculates with spermatozoa having low percentage motility (24), subnormal forward velocities (23) and low motility indices (25) are less likely to fertilize ova. The fact that these three vital parameters of motility were concomitantly impaired by enalapril makes it, or a derivative, worth investigating further as a vaginal contraceptive. Ideally, such a derivative should be, compared to enalapril, less easily reversed by washing in, for example, cervical fluid.

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REFERENCES


CONTRACEPTION


