Effects of co-trimoxazole, erythromycin, amoxycillin, tetracycline and chloroquine on sperm function in vitro

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This in-vitro study was designed to investigate the effects of commonly prescribed antibiotics on sperm movement characteristics, viability and the ability of spermatozoa to undergo the acrosome reaction. Spermatozoa were obtained by swim-up from normozoospermic semen and cultured for 24 h with increasing concentrations of co-trimoxazole, erythromycin, amoxycillin, tetracycline and chloroquine. Tetracycline at concentrations as low as 2.5 µg/ml led to a significant dose-dependent inhibition in percent rapid-moving spermatozoa, mean path velocity (VAP), straight-line velocity (VSL) and curvilinear velocity (VCL), but at 50 µg/ml tetracycline all spermatozoa were static. Erythromycin had significant effects on rapid movement, VAP, VSL and VCL only at concentrations >100 µg/ml. In contrast, percent rapid-moving spermatozoa was significantly enhanced at low concentrations of chloroquine (5 µg/ml), but significantly inhibited by higher concentrations. Co-trimoxazole did not adversely affect percent rapid-moving spermatozoa below 500 µg/ml, at which concentration movement was decreased by 34%. The mean lateral head displacement (ALH) was significantly enhanced by 5 µg/ml co-trimoxazole and reduced at 1 mg/ml erythromycin. The effects of these drugs were mostly irreversible. Amoxycillin had no effect on sperm movement characteristics over the dose range used, though it inhibited viability at high doses. Viability was significantly reduced at concentrations of all drugs which affect rapid sperm movement; these concentrations of drugs did not appear to affect the ability of spermatozoa to undergo the acrosome reaction. The results from this study, when combined with known effects on spermatogenesis, should facilitate the choice of drugs for the treatment of both genitourinary and unrelated infections in men who are attempting to conceive.

Key words: acrosome/antibiotics/chloroquine/male fertility/spERM movement

Introduction

Approximately 50% of known causes of primary infertility are attributed to male factor (Yates et al., 1989); however, the aetiology of male factor infertility is not easy to define. While it is becoming increasingly clear that certain individuals may be genetically predisposed to being subfertile (Reijo et al., 1996), there are many epigenetic factors which have been implicated as potential causes of male infertility. Environmental pollutants as well as modern-day social habits such as smoking, consumption of alcohol and drug taking have all been associated with male subfertility (Marshburn et al., 1989; Schlegel et al., 1991; Sharpe and Skakkebaek, 1993; Tas et al., 1996). Certain chronic systemic illnesses are known to impair fertility potential and some bacterial or viral diseases which may or may not be associated with the male reproductive tract may also adversely affect semen parameters.

Antibiotics such as amoxycillin and tetracycline are commonly prescribed for a multitude of everyday conditions. Not surprisingly, a proportion of male patients attending fertility clinics may have been prescribed antibiotics by their general practitioner to treat these unrelated infections. In addition, some patients requiring assisted conception occasionally show evidence of infection of the male reproductive tract. The antibiotics co-trimoxazole and erythromycin are routinely used by urologists, andrologists and fertility specialists to treat such bacterial infections occurring prior to in-vitro fertilization (IVF) treatment, or when high concentrations of leukocytes are present in the semen of these patients, irrespective of microbial evidence of infection. In our experience, patients on a course of antibiotics often demonstrate below-average semen parameters. While in some instances this may be caused by the infection itself, it is likely that the antibiotics have a direct effect on sperm function.

For an antibiotic to be effective and safe for treating conditions in the male who wishes to conceive, it must satisfy two criteria. First, it must have a bacteriocidal effect against the relevant pathogen, and second, it must have no impact on spermatogenesis and mature sperm function. Studies in mammals and humans have shown that antibiotics from all the major classes have significant adverse effects on spermatozoa. Antibiotics co-trimoxazole and erythromycin are routinely used by urologists, andrologists and fertility specialists to treat such bacterial infections occurring prior to in-vitro fertilization (IVF) treatment, or when high concentrations of leukocytes are present in the semen of these patients, irrespective of microbial evidence of infection. In our experience, patients on a course of antibiotics often demonstrate below-average semen parameters. While in some instances this may be caused by the infection itself, it is likely that the antibiotics have a direct effect on sperm function.

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A successful pregnancy, whether achieved through natural conception or as a result of assisted conception, is dependent upon the normal function of the gametes. Therefore it is imperative that the effects of antibiotics on sperm function are

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known when treating patients for an infection prior to IVF treatment, or those in the general public who wish to conceive. This study was therefore conducted to determine whether commonly prescribed antibiotics, within the therapeutic dose range, are detrimental to mature sperm function in vitro. The antimalarial drug chloroquine was also investigated, as it has been reported to severely inhibit sperm motility and subsequent fertility in rats (Okanlawon et al., 1993). The results may assist in the choice of drugs which can be prescribed safely without affecting potential fertility.

Materials and methods

Sperm preparation

Semen was obtained from patients attending for routine semen analysis. Only semen demonstrating parameters within the normal range, for count, motility and abnormal forms as described by the World Health Organization (1992) was used. Semen was washed with an equal volume of Earle’s balanced salt solution (EBSS; Life Technologies, Ltd, Paisley, UK) containing 1 mM sodium pyruvate, Sigma, Poole, UK, 25 mM NaHCO3; BDH, Essex, UK, supplemented with 10% Albuminar-5 (containing 5% human serum albumin; Armour Pharmaceutical Co., Eastbourne, UK). Following centrifugation at 300 g for 10 min, the supernatants were discarded, and 1 ml medium layered on top of the pellets. The motile spermatozoa were allowed to swim-up at 37°C under 5% CO2 in air for 60 min. The supernatants were examined on a Makler chamber and the spermatozoa concentration adjusted to approximately 10×106 spermatozoa/ml.

Dose–response

Aliquots of spermatozoa (0.5 ml) were cultured for 24 h with increasing concentrations of antibiotic. Erythromycin (Abbott Laboratories Ltd, Maidenhead, UK), co-trimoxazole (The Wellcome Foundation Ltd, Manchester, UK), amoxycillin (SmithKline Beecham Pharmaceuticals, Weybridge, UK), chloroquine (Rhone-Poulenc Rorer Ltd, Eastbourne, UK) and tetracycline (Ledeler Laboratories, Gosport, UK) were all preparations for injection without any preservatives. Stock solutions of chloroquine and co-trimoxazole were as follows: chloroquine, 40 mg/ml; co-trimoxazole (1 part trimethoprim to 5 parts sulphamethoxazole) 96 mg/ml. Stock concentrations of drugs which were purchased not in solution were prepared using water for injection and were as follows: tetracycline and erythromycin, 50 mg/ml; amoxycillin, 500 mg/ml. For experiments, stock solutions of all drugs were diluted with 10% Albuminar-5 in culture medium as described above, and added to spermatozoa suspensions in no more than 50 µl/ml. Controls were cultured in the absence of antibiotic for the same period of time. Spermatozoa were placed on Camlab (Cambridge, UK) microslides at 200 µm depth. With this depth, sperm suspensions separated into three distinct layers: highly motile (upper), small number motile (middle) and cross-section (lower). Care was taken to ensure that the lower layer of spermatozoa, consisting of a cross-section of the sperm population, was consistently selected as the field of view for motility analysis. A detailed analysis of sperm movement characteristics was performed using a Hamilton Thorn motility analyser (Microm UK Ltd, Thame, UK) at 60 frames/s at 37°C. It should be noted that fast downward-swimming spermatozoa would have been excluded from the analysis since they immediately moved out of the focal plane. The computer-assisted semen analysis (CASA) settings were minimum contrast, 45; minimum cell size, 3; low and high static head size gates, 0.48 to 2.91; low and high static head intensity gates, 0.48 to 1.80; non-motile head intensity, 130; static elongation 0 to 100 frames acquired 30 and 1.95 magnification. The ‘play back’ facility was utilized at the end of each analysis to ensure that track acquisition was optimal and, if necessary, the gate settings were changed accordingly.

Reversibility of effects

To determine whether any of the effects observed in the treatment groups were reversible, 0.5 ml aliquots of washed spermatozoa were cultured in tubes for only 4 h with the drug at 37°C. After this time, 4 ml culture medium was added to each tube, which was then centrifuged at 250 g. The supernatants were discarded and the spermatozoa pellets resuspended in fresh culture medium in the absence of antibiotic. Culture was then continued for a further 20 h without antibiotic, after which time sperm motility parameters were compared. The concentration of drug used was that which reduced rapid motility by approximately 50%; 25 µg/ml tetracycline, 50 µg/ml chloroquine and 500 µg/ml erythromycin and co-trimoxazole. Controls were similarly cultured for 4 h, washed and recultured in the continuous absence of drug.

Acrosome reaction assay

Motile sperm preparations were adjusted to 5×106 cells/ml and cultured for 24 h at 37°C with concentrations of drugs sufficient to cause an effect as determined in the previous experiments. The spermatozoa were then sedimented by centrifugation at 200 g, resuspended in fresh medium in the absence of drug, and separated into two aliquots. The first aliquot was used as a measure of the sperm population that would undergo acrosomal exocytosis after stimulation by the calcium ionophore A23187 (free acid; Sigma, Poole, UK). The sperm suspension was incubated with 5 mM ionophore dissolved in 1 ml dimethyl sulphoxide (BDH) for 60 min at 37°C. The second aliquot acted as a measure of the spontaneous acrosome exocytosis exposed to the solvent only, in the absence of ionophore. Following incubation, sperm suspensions were centrifuged at 200 g for 5 min to remove the ionophore and incubated for a further 60 min at 37°C in 0.5 ml hypo-osmotic swelling solution (WHO, 1992). The sperm suspensions were re-centrifuged (200 g) for 5 min and the pellets resuspended in 50 ml ice-cold ethanol. The slides were prepared by smearing 10 µl ethanol-sperm suspension over a clean standard microscope slide and allowing the smear to air-dry at room temperature. The acrosome status was determined on fixed cells using the fluorescent probe fluorescein isothiocyanate (FITC)-conjugated peanut lectin (Arachis hypogaea); Sigma. This lectin is directed to the outer acrosomal membrane and was selected in preference to Pisum sativum which targets the acrosomal vesicle contents, as it is consistently more efficient (Aitken and Brindle, 1993). Both spontaneous acrosome exocytosis as well as calcium ionophore A23187-induced acrosome exocytosis was determined. Acrosomal status was assessed on a Leitz 100Z microscope equipped with phase contrast and epifluorescence optics. The Hg excitation beam was passed through a 340–380 nm bandpass filter and fluorescence emission was observed through an RKP 400 beam-splitting mirror. Some 200 live cells were assessed for acrosomal status. Spermatozoa that were observed with fluorescence in an even uniform pattern over the anterior portion of the head were considered to be acrosome-intact. Spermatozoa with a single fluorescent band in the equatorial segment were considered acrosome-reacted. An intermediate stage was observed where the fluorescence was patchy in the anterior portion of the head with a single bright band of fluorescence in the equatorial segment. These forms were excluded from the acrosome-reacted category.
Figure 1. Effects of increasing concentrations of chloroquine, erythromycin, amoxycillin, tetracycline and co-trimoxazole on the proportion of rapid and static spermatozoa. The spermatozoa from one semen sample from one individual was used to determine an entire dose–response to one drug only. Each dose–response curve represents the mean values from three to four individuals on separate days. Paired t-tests were carried out comparing values at each concentration to the value in the absence of drug (0 µg/ml) for each individual’s sperm preparation.

Viability
The World Health Organization (1992) method for assessing the viability of the spermatozoa was used. A 50 µl aliquot of sperm suspension and 50 µl of the Eosin–Nigrosin stain (WHO, 1992) were thoroughly mixed in a microfuge tube and gently smeared onto ethanol-cleaned microscope slides. The slides were allowed to air-dry for 30 min and mounted with DPX mounting medium (Sigma) in a fume cupboard. The percentage of viable cells, that excluded the dye, was calculated from an assessment of 200 cells. A variety of fields were viewed using ×400 magnification and bright field microscopy.

Statistics
Statistics were performed using Astute Statistics Software (DDU Software, Leeds, UK). The paired t-test was used to compare sperm motility at each antibiotic dose with the control (no antibiotic). Differences were considered significant if the probability of the events occurring due to chance were < 0.05.

Results
Effect of antibiotics on sperm movement parameters
The results showed that different antibiotics have varying effects on sperm motility. Figure 1a and c shows the proportion of spermatozoa moving rapidly (motility of >25 mm/s) after 24 h incubation, while Figure 1b and d indicate the proportion of spermatozoa that are static. Amoxycillin had no significant effect on either of these parameters over the concentration range tested (Figure 1a and b). Chloroquine had a dual effect, enhancing rapid motility at low concentrations (5 µg/ml, 62.3% rapid versus 48.0% in the absence of antibiotic, P = 0.04), but inhibiting it at higher concentrations (100 µg/ml, 10% rapid, P = 0.0007) (Figure 1a). At 250 µg/ml chloroquine, all spermatozoa were static (Figure 1b). At 100 µg/ml erythromycin, there was a significant decline in rapid moving spermatozoa (Figure 1c) from 71.8% to 52.3% (P = 0.049). At 25 µg/ml chloroquine, all spermatozoa were static (Figure 1b). At 100 µg/ml erythromycin, there was a significant decline in rapid moving spermatozoa (100 µg/ml, 44.3% rapid versus control, 58.0% rapid; P = 0.032) (Figure 1a) which was enhanced at higher concentrations (500 µg/ml, 33.9% rapid, P = 0.007 and 1 mg/ml, 18.0% rapid, P = 0.009). This was accompanied by an increase in slow moving sperm (data not shown) until 2 mg/ml, where almost all spermatozoa were static (Figure 1b). The spermatozoa were particularly sensitive to tetracycline because doses as low as 2.5 µg/ml significantly reduced the proportion of rapidly moving spermatozoa (Figure 1c) from 71.8% to 52.3% (P = 0.049). At 25 µg/ml, only 9.5% spermatozoa were rapidly moving (P = 0.003) and over 50%
Antibiotics and sperm function

Figure 2. Effects of increasing concentrations of drugs on the mean path velocity (VAP) and mean straight-line velocity (VSL) of cultured spermatozoa. For details, see legend to Figure 1.

were static \((P = 0.02)\) (both compared with the absence of drug); at 50 \(\mu\)g/ml tetracycline, all spermatozoa were static (Figure 1d). By contrast, only high concentrations of co-trimoxazole (500 \(\mu\)g/ml) impaired rapid sperm movement (Figure 1c).

On examining the movement characteristics in more detail, amoxycillin had no significant effect on either the straight-line velocity (VSL) or the mean path velocity (VAP) (Figure 2a and b). Interestingly, while concentrations of chloroquine \(\geq 50\ \mu\)g/ml significantly reduced the percentage of rapid sperm, they had no significant effect on either velocity parameter. In contrast, concentrations of erythromycin which decreased rapid motility also significantly impaired both velocity parameters (0 versus 100 \(\mu\)g/ml: VAP, \(P = 0.013\) and VSL, \(P = 0.005\)). In addition, tetracycline significantly reduced both VAP \((P = 0.045)\) at 10 \(\mu\)g/ml and VSL at 25 \(\mu\)g/ml \((P = 0.022)\) (Figure 2c and d).

Analysis of the curvilinear velocity (VCL) (Figure 3a and b) showed a significant decline at 1 mg/ml erythromycin \((P = 0.017)\) and 25 \(\mu\)g/ml tetracycline \((P = 0.036)\), while amoxycillin, chloroquine and co-trimoxazole each had no effect. The mean linear head displacement (ALH) was significantly reduced at 1 mg/ml erythromycin \((P = 0.009)\) (Figure 3c), but was significantly increased at low concentrations of co-trimoxazole compared with the control (0 versus 5 \(\mu\)g/ml; \(P = 0.03\)) (Figure 3d). The observed decrease in ALH at 25 \(\mu\)g/ml tetracycline was not significant (Figure 3d), even though this concentration significantly impaired sperm velocity characteristics.

Reversibility studies

To determine whether the effects on sperm movement were reversible, spermatozoa were cultured for 4 h in the presence of drugs at concentrations which had reduced the percent rapid-moving sperm by >50\%, as determined in the previous experiment. Spermatozoa were then washed and resuspended in culture medium in the absence of drug and cultured for an additional 20 h. The effects of the drugs on sperm motility were mainly irreversible, as the drugs continued to exert their effects 20 h after their removal from the culture medium (Figures 4 and 5). Results showed that, after only 4 h (hatched bars), 50 \(\mu\)g/ml chloroquine and 500 \(\mu\)g/ml erythromycin and co-trimoxazole were already taking effect, significantly reducing the percent rapid-moving sperm (Figure 4a) and increasing levels of static sperm (Figure 4b) compared with the control \((P = 0.004, 0.002\) and 0.004, respectively for \% rapid-moving, and 0.007, 0.002 and 0.028 for \% static). Again, this was reflected in a significant
Figure 3. Effects of increasing concentrations of drugs on the mean curvilinear velocity (VCL) and mean lateral head displacement (ALH) of cultured spermatozoa. For details, see legend to Figure 1.

decrease in VAP (Figure 5a), VSL (Figure 5b) and VCL (Figure 5c) compared with controls, while ALH remained unaffected by any drugs at this time (Figure 5d). Interestingly, tetracycline appeared to have relatively little effect on sperm parameters tested after only 4 h incubation. Only rapid motility (Figure 4a) and VSL (Figure 5b) were significantly reduced at this time (tetracycline versus control percent rapid-moving; \( P < 0.029 \); VSL, \( P = 0.003 \)).

If the drugs were then washed out and incubation continued in their absence (filled bars in Figures 4 and 5) for a total of 20 h, the difference in percent rapid-moving spermatozoa between treatment groups and control became even more enhanced, accompanied by a corresponding increase in percent static sperm. This occurred with all drugs tested (Figure 4a and b). This enhanced adverse effect on sperm movement characteristics was also reflected in the VAP, VSL and VCL (Figure 5a–c). All these parameters were more impaired after 20 h following brief exposure to tetracycline, erythromycin, chloroquine or co-trimoxazole. Interestingly, ALH was not significantly altered by any drug after 4 h incubation, though when drugs were washed out, continuation of incubation in medium without drug led to a reduction in ALH following brief exposure to erythromycin and co-trimoxazole (\( P = 0.05 \) and 0.042, respectively) (Figure 5d).

Sperm viability
Sperm viability was determined following overnight culture with the same concentrations of drug as used in the reversibility study. In addition, the effect of 1 mg/ml amoxycillin was investigated. At drug concentrations which impaired sperm movement characteristics, tetracycline (25 µg/ml), chloroquine (50 µg/ml), erythromycin (500 µg/ml) and co-trimoxazole (500 µg/ml) also caused a significant decrease in sperm viability (Table I). In addition, 1 mg/ml amoxycillin—which has no effect on sperm movement—significantly reduced the percent-age of viable sperm compared with control following 24 h incubation with the drug.

Acrosome reaction
The effects of the drugs on the ability of spermatozoa to undergo the acrosome reaction was also determined (Table II). Results are presented in terms of the acrosome reaction with ionophore challenge (ARIC) score, which is the difference between the percentage of A23187-induced acrosome-reacted spermatozoa and the percentage of spontaneously acrosome-reacted spermatozoa. There was a tendency for tetracycline, amoxycillin—and particularly co-trimoxazol—to impair the acrosome reaction, though not signific-
three times using semen from three individuals. Paired reversibility of all of the drugs. The experiment was carried out bars). Spermatozoa from one individual were used to test the culture continued for a further 20 h in its absence. Sperm (hatched bars). Spermatozoa were washed free of the drug and absence of drug. After 4 h, movement characteristics were assessed Control samples were incubated under the same conditions in the 50 \( \mu g/ml \) chloroquine (Ch) or 500 \( \mu g/ml \) co-trimoxazole (Co-t). Control samples were incubated under the same conditions in the absence of drug. After 4 h, movement characteristics were assessed (hatched bars). Spermatozoa were washed free of the drug and culture continued for a further 20 h in its absence. Sperm movement characteristics were assessed again at this time (filled bars). Spermatozoa from one individual were used to test the reversibility of all of the drugs. The experiment was carried out three times using semen from three individuals. Paired \( t \)-tests were carried out to determine differences between the treated groups and their corresponding controls after the same incubation period. *\( P < 0.05 \); **\( P < 0.005 \).

antly so. When analysing the raw data, it was apparent that the spermatozoa of some individuals were sensitive to the drugs, while others were not.

Discussion
In this study it has been shown that tetracycline, chloroquine, erythromycin and co-trimoxazole considerably impair sperm movement characteristics and significantly reduce sperm viability in vitro. These effects are irreversible. While the mechanism of action of these drugs in terms of inhibition of protein synthesis, impairment of folic acid synthesis and intercalation with DNA is well documented (Gilman et al., 1985), it is highly likely that there are other non-specific effects which are contributing to impairment of sperm motility.

Over the concentration range used, amoxycillin is the least toxic of all the drugs tested, although at high concentrations it also decreased sperm viability. The negligible effects of amoxycillin found in this study confirm the observation by White (1954) that penicillins do not adversely affect progressive sperm motility at concentrations of up to 5 \( g/ml \). The mode of action of the penicillin family of drugs is by interference with the structural integrity of the bacterial cell wall, resulting in cell lysis; thus, they would not be expected to affect mammalian cells. However the penicillin derivative, \( \beta \)-penicillamine, which acts as a divalent cation chelator, has a significant effect on mature sperm function as it is able to induce capacitation of hamster spermatozoa (Andrews and Bavister, 1989).

The ability of spermatozoa to undergo the acrosome reaction is not significantly impaired in the presence of any of the drugs tested. Tetracycline would be expected to inhibit the acrosome reaction as it is a very effective chelator of calcium which is required for the acrosome reaction to occur. However, the interpretation of the acrosome reaction in the presence of tetracycline is unclear, as this antibiotic is a known fluorophore and binds avidly to human spermatozoa (Ericsson and Baker, 1967), particularly around the midpiece region, and thus may interfere with the assay.

Tetracycline is the most potent drug tested as significant effects on sperm movement have been seen at concentrations as low as 2.5 \( \mu g/ml \), well within those achieved following therapeutic doses of the antibiotic. This is in contrast to chlortetracycline, which only affected progressive motility of spermatozoa in vitro at concentrations above 100 \( \mu g/ml \) (White, 1954). Tetracycline and its derivatives are often prescribed for infections of the genitourinary tract and within 2–4 h of a single, 250 mg oral dose, plasma concentrations are approximately 3 \( \mu g/ml \) (Gilman et al., 1985) and rise to approximately 7 \( \mu g/ml \) following a single i.v. dose of 500 mg (Borski et al., 1954). Because there is excellent penetration of tetracyclines into prostatic fluid and particularly semen, with concentrations about 60% of those found in serum (Borski et al., 1954), it is highly likely that the effects seen on mature spermatozoa in vitro would be reflected in vivo during a course of tetracycline treatment. Tetracyclines inhibit protein synthesis, yet surprisingly they appear to be relatively non-toxic to spermatogenesis (Timmermans, 1974; Kushniruk, 1976), their effects being more pronounced on mature sperm function. It is not clear how tetracycline impairs sperm motility; however, its ability to chelate calcium may be highly relevant because calcium is not only involved in initiating mature mammalian sperm movement but is also crucial for hyperactivation (Yanagamachi, 1994). Additionally, binding of the drug to the sperm may actually present a physical obstruction to motility.

Unlike the tetracyclines, co-trimoxazole treatment of rats leads to a significant impairment of spermatogenesis (Crotty et al., 1995). Co-trimoxazole is a combination of a sulphamethoxazole with trimethoprim (5 parts to 1 part), which
Sulphur drugs such as sulphasalazine have been used for many years as a treatment for ulcerative colitis or Crohn’s disease, and they are known to impair fertility. Sulphasalazine leads to oligospermia, poor sperm motility and morphological changes accompanied by a poor pregnancy rate (Toovey et al., 1981; Cosentino et al., 1984). Sulphamethoxazole alone significantly reduces progressive motility of human spermatozoa in vitro only at exceptionally high concentrations (5 mg/ml; White, 1954) while in the current study, it has been shown that the combination with trimethoprim increases the sensitivity of spermatozoa to the drug approximately 10-fold. Co-trimoxazole is absorbed efficiently into prostatic fluid and is thus widely used for treating genitourinary infection. Following one week of co-trimoxazole treatment, concentrations of trimethoprim in prostatic tissue rise considerably compared with plasma levels (8.2 µg/g and 4 µg/ml, respectively), whereas sulphamethoxazole is not absorbed to the same extent (48.7 µg/ml in plasma and 17 µg/g in prostatic tissue) (Dabhiowala et al., 1976). At 2 h following an oral dose of co-trimoxazole, semen concentrations of trimethoprim range from 1.2 to 9.1 µg/ml compared with <20 to 74 µg/ml for sulphamethoxazole (Eliasson and Dornbusch, 1977). As these concentrations are below those found to have a pronounced adverse effect on sperm function in vitro, co-trimoxazole treatment would not be expected to hamper mature sperm function. However, it is together interfere with folic acid synthesis and metabolism.

<table>
<thead>
<tr>
<th>Drug (concentration)</th>
<th>Mean^ viability (n = 25)</th>
<th>P (versus control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>44.7 ± 2.0</td>
<td>–</td>
</tr>
<tr>
<td>Co-trimoxazole (500 µg/ml)</td>
<td>31.8 ± 2.5</td>
<td>&lt; 0.0002</td>
</tr>
<tr>
<td>Tetracycline (25 µg/ml)</td>
<td>28.0 ± 2.4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Aminocillin (1 mg/ml)</td>
<td>38.4 ± 1.8</td>
<td>&lt; 0.029</td>
</tr>
<tr>
<td>Erythromycin (500 µg/ml)</td>
<td>30.8 ± 1.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Chloroquine (50 µg/ml)</td>
<td>27.9 ± 1.9</td>
<td>&lt; 0.0001</td>
</tr>
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^Values are mean ± SEM.

<table>
<thead>
<tr>
<th>Drug (concentration)</th>
<th>Mean^ ARIC score (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>19.10 ± 3.78</td>
</tr>
<tr>
<td>Co-trimoxazole (50 µg/ml)</td>
<td>14.25 ± 2.09</td>
</tr>
<tr>
<td>Tetracycline (25 µg/ml)</td>
<td>16.75 ± 2.10</td>
</tr>
<tr>
<td>Aminocillin (1 mg/ml)</td>
<td>16.08 ± 3.36</td>
</tr>
<tr>
<td>Erythromycin (500 µg/ml)</td>
<td>22.85 ± 2.50</td>
</tr>
<tr>
<td>Chloroquine (50 µg/ml)</td>
<td>18.85 ± 3.36</td>
</tr>
</tbody>
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^Values are mean ± SEM.

ARIC = acrosome reaction with ionophore challenge.

None of the drugs had a statistically significant effect when compared with the controls.
Taking long-term chloroquine may be subjected to tissue damage. Thus, it is plausible that spermatozoa in individuals who receive chloroquine treatment for one month with co-trimoxazole in men with bacteriological-positive culture (Merino and Carranza-Lira, 1995). Another study investigating the treatment of bacterial infections in 40 infertility patients, revealed a decrease in sperm count in 37% of patients receiving drug treatment for 14–17 days, while 42% actually showed an improvement in count (Murdia et al, 1978). Interpretation of all these studies is complicated by the fact that it is unclear whether the alterations in semen parameters are attributable to either the presence or the eradication of the infection, or whether the patient’s own inflammatory response had contributed to the poor semen parameters in the first instance. Furthermore, it is not stated whether all of the patients actually responded to the treatment. Clearly, whatever the effects of co-trimoxazole on mature sperm function, the inhibitory effects of this antibiotic on spermatogenesis (Crotty et al., 1995) must also be taken into consideration when selecting this drug for treatment.

Erythromycin is a macrolide antibiotic which was also shown to impair sperm function in vitro at relatively high concentrations. Erythromycin functions by inhibiting protein synthesis and has previously been shown to impair mitosis in rat spermatids, although the effects are reversible (Lastikka et al., 1976). This is in contrast to the effects reported in this study where the impairment of mature sperm function was not reversible. In support of these observations, short-term exposure of macrolides to human spermatozoa impairs motility and is spermicidal at high doses (1 mg/ml; White, 1954). Erythromycin diffuses freely into prostatic fluid (Borski et al., 1954) to concentrations approximating 40% of those found in plasma, and reaching a maximum of 2 µg/ml after a single oral dose of 500 mg (Gilman et al., 1985). Thus, it is probably unlikely that erythromycin levels would accumulate sufficiently in semen to affect mature sperm function during a routine course of treatment. Josamycin, another macrolide antibiotic, is absorbed into prostatic fluid and seminal vesicle fluid to approximately the same concentration as that found in serum (2.23 µg/ml and 1.56 µg/ml). Interestingly, concentrations as low as 0.5 µg/ml josamycin actually improved human sperm motility in vitro, although it is unclear whether progressive motility was measured (Schrann et al., 1988).

In the present study, there was also a beneficial effect on the percent of rapidly moving sperm in the presence of low concentrations of chloroquine (5 µg/ml) and which was in contrast to the adverse effects seen at ten times this concentration. Chloroquine is almost completely absorbed into the bloodstream and is concentrated in the tissues, with concentrations being 200- to 700-fold those in plasma (Gilman et al., 1985). Thus, it is plausible that spermatozoa in individuals taking long-term chloroquine may be subjected to tissue damage. Chloroquine concentrations approaching those that are capable of producing adverse effects on sperm function were difficult to draw any conclusive evidence about the effects of this antibiotic on semen parameters in vivo, as the results from such studies are conflicting. While co-trimoxazole treatment of urologic and dermatologic patients had no short-term effect on sperm motility, morphology and count, a significant adverse effect on all these parameters occurred four weeks post treatment (Lange and Schirren, 1974). In contrast, another study showed no alteration in these parameters following treatment for one month with co-trimoxazole in men with bacteriological-positive culture (Merino and Carranza-Lira, 1995).

References

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