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Susceptibility of Glycolytic Enzyme Activity and Motility of Spermatozoa From Rat, Mouse, and Human to Inhibition by Proven and Putative Chlorinated Antifertility Compounds In Vitro

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ABSTRACT: Nonhormonal contraceptives that act by blocking energy metabolism within sperm have the advantage over spermatogenic inhibitors by their fast onset of infertility and their almost immediate restoration of fertility after withdrawal of the contraceptive agent. This study was done to test new chlorinated compounds for their contraceptive potency on rodent and human sperm in vitro. Cells were incubated in a medium containing glucose as the sole energy source with 1-chloro-3-hydroxypropanone (CHOP) and 1,6-dichloro-1,6-dideoxy-D-fructose (DCDF), chlorinated analogues of glycolytic substrates, as well as racemic (R,S)-α-chlorohydrin (ACH). After incubation, enzymatic activity and kinematic parameters were estimated. A dose-dependent inhibition of the glycolytic enzyme, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), of rat and mouse distal cauda epididymidal and human ejaculated sperm by ACH, CHOP, and DCDF was demonstrated. Triosephosphate isomerase (TPI) was inhibited by ACH, but not by CHOP and DCDF, irrespective of species. All compounds inhibited sperm motility and kinematic parameters with increasing concentration. The results confirm that inhibition of glycolytic enzymes of sperm, including those of human, can be effectively brought about by a variety of chloro-compounds that can be converted to (S)-3-chlorolactaldehyde, the stereospecific chloro-derivative of the enzyme's natural substrate, (R)-glyceraldehyde 3-phosphate, and could be developed into contraceptive agents for men.

Key words: Chlorinated compounds, antifertility agents, rat, ornidazole, α-chlorohydrin, chlorohydroxypropanone, dichlorodideoxyfructose.

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New methods of contraception for men could be one answer to the increasing world population that degrades the quality of life and the environment, and which demands drastic measures to curtail. The hormonal contraceptive approach for men, in which spermatogenesis is blocked, has reached an advanced stage, but the production of ''functional sterility'' by inhibiting sperm function, without necessarily killing them, is also promising. This type of contraception is advantageous because the onset of infertility after administration and the reversibility of such contraceptives are rapid (Cooper and Yeung, 1999). One example of this is the reduction in adenosine 5′-triphosphate (ATP) production in sperm by chlorinated 3-carbon compounds or derivatives of these compounds. Although there are species differences in the ways in which sperm ATP is derived, with human sperm deriving 80% from substrate-level phosphorylation and rats deriving 95% from respiration (Ford and Rees, 1990), a shift to glycolysis is observed during capacitation in the rat so that irreversible blockage of glycolysis may have effects on fertilization-related events. For example, ornidazole, a chlorinated nitroimidazole that inhibits sperm glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and triosephosphate isomerase (TPI) after oral administration to male rats, induces infertility by natural mating (Oberländer et al, 1994). However, sperm from these males do not undergo hyperactivation and cannot penetrate the zona pellucida of oocytes in vitro even when they are supplied with lactate (Bone et al, 2000).

The immediate product of ornidazole metabolism is unknown, although Jones and Cooper (1997) identified 3-chlorolactate in the urine of male rats after administration of ^{36}Cl-ornidazole, which suggests side-chain cleavage to chlorolactaldehyde, α-chlorohydrin, or 1-chloro-3-hy-

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droxypropanone (CHOP). Both (R,S)-3-amino-1-chloropropanol (Jones et al, 1979) and 6-chloro-6-deoxyglucose (Jones and Dobbie, 1991) are metabolized in the rat to (R,S)-α-chlorohydrin or 3-chlorolactaldehyde as determined by the analysis of urinary metabolites. Thus, contraceptive activity is associated with the production of inhibitors of GAPDH, generated either within sperm or by other organs and transferred to the epididymis from blood.

(R,S)-α-Chlorohydrin, at low doses not causing epididymal obstruction, was considered an ideal contraceptive agent for rodents in the 1970s, although some aspects of its action (exacerbation of its toxicity by glucose) remain unclear (Ford and Harrison, 1985, 1986, 1987). However, administration to macaques was reportedly followed by damage to their bone marrow (Kirton et al, 1970; Setty et al, 1970), although the purity of the compounds administered has been questioned (Jones and Cooper, 1999). Nevertheless, the neural effects produced by both (S)-α-chlorohydrin and 6-chloro-6-deoxyglucose in mice and marmosets (Ford and Waites, 1982) highlight the dangerous side effects that such compounds could present. These effects could be obviated by using more effective compounds that could be provided at lower doses. Other compounds that are reported to produce the same inhibitory metabolite as ACH, such as CHOP (Cooper and Jones, 2000) and DCDF (Jones and Morin, 1995; see Fig. 1) may be just as effective as (R,S)-α-chlorohydrin at inhibiting sperm enzymes with fewer side effects.

The clinical relevance of the animal studies for male contraception was questioned by Homonnai et al (1975), who reported that 300 mmol/L of (R,S)-α-chlorohydrin reduced human sperm motility only by 40% in vitro, in contrast to 1 mmol/L in rats (Bone and Cooper, 2000). This raises the question of whether human sperm are generally less susceptible to inhibition by chlorinated 3-carbon compounds. The present study was done to compare the effectiveness of (R,S)-α-chlorohydrin and other chlorocompounds in vitro on the motility, as an indicator of glycolytic turnover in lactate-free and pyruvate-free medium, and glycolytic enzyme activity of sperm from rats, mice, and men. A preliminary report of the effects of these compounds on human sperm has been presented in abstract form (Cooper et al, 2000).

Materials and Methods

Chemicals and Animals

Chemicals were obtained from Sigma (Deisenhofen, Germany) at the purest grade available. Adult (250–550 g) male Sprague-Dawley CD rats and 5-week old male mice (20–22 g) of the CB6F1/Crl BR hybrid strain were obtained from Charles River Wiga GmbH (Sulzfeld, Germany) and held under conditions of

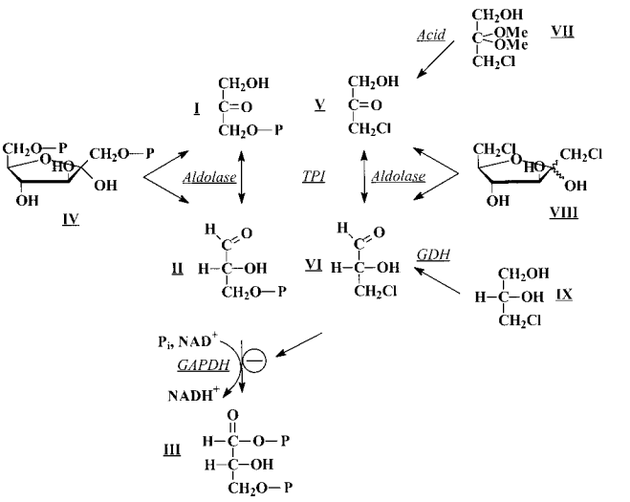

Figure 1. Sperm metabolic pathways of putative antifertility agents demonstrated in the boar and suspected for rats. On the left side is the normal glycolytic pathway in which dihydroxyacetone phosphate (DHAP, I) is converted by flagellar TPI to (R)-glyceraldehyde-3-phosphate (GAP, II). This is further converted by flagellar GAPDH to 1,3-diphosphoglycerate (DPGA, III), which provides a high-energy phosphate to adenosine diphosphate in substrate-level phosphorylation. Fructose-1,6-bisphosphate (IV) yields both I and II by the action of aldolase. The analogous chlorinated compound to DHAP, CHOP (V), is converted by TPI to (S)-3-chlorolactaldehyde (VI), which cannot be converted by GAPDH, and therefore blocks energy production from its normal substrate. The stable precursor of V, its dimethylketal (VII), is hydrolyzed by low pH. 1,6-Dichloro-1,6-dideoxyfructose (VIII) is cleaved by aldolase to both V and VI. α-Chlorohydrin (IX) requires a cytoplasmic $NADP^+$-dependent glycerol dehydrogenase (GDH) to be converted to VI. P indicates the phosphate group.

12 hours of light and 12 hours of dark (lights on 0700 hours) at 22°C and 50% relative humidity with access to standard chow (Altrumin GmbH, Lage, Germany) and water ad libitum. (R,S)-α-Chlorohydrin was vacuum-distilled twice before use, CHOP was prepared as described by Jones et al (1986), and 1,6-dichloro-1,6-dideoxy-D-fructose (DCDF) by the procedure of Jones and Morin (1995).

Collection of Cauda Epididymidal Spermatozoa from Rats and Mice

Rat sperm were collected as described by Bone and Cooper (2000). Briefly, sperm were obtained from the distal cauda epididymidis of rats by cannulating the vas deferens, cutting the mid-cauda epididymidis, and flushing out the luminal contents with equilibrated medium G, which contains glucose as a sole energy source (Bone et al, 1997; osmolality, 310 ± 5 mmol/kg). Sperm were collected in 1 mL of equilibrated medium G (37°C, 5% CO_2) and were kept for at least 10 minutes in an incubator. After dispersion, concentration was assessed by nephelometry (Bone and Cooper, 2000).

Up to 3 male mice per experiment were asphyxiated with CO_2, the epididymides removed through a scrotal incision, and each cauda epididymidis transferred to a Petri dish containing 700 μL of equilibrated medium G under paraffin oil. Each caudal tubule was punctured with needles, the sperm suspension was allowed

to disperse over 30 minutes at 37°C, and the concentration was assessed by nephelometry.

Human Spermatozoa

Ejaculates were obtained from 14 normozoospermic men (28–44 years of age) attending the Institute of Reproductive Medicine. After liquefaction, spermatozoa were washed free of seminal plasma by the addition of 5 volumes of medium G and centrifuged at 500 × *g* for 7 minutes at room temperature (Heraeus; Hanau, Germany). After recovery of the pellets, 5 mL of medium G were added and the sperm suspension was mixed and centrifuged at 400 × *g* for 5 minutes at room temperature. The pellet obtained was dispersed in 1 mL of medium G in an incubator, and sperm concentrations were ascertained by hemocytometry (World Health Organization, 1999).

Incubation of Sperm with (R,S)-α-Chlorohydrin, CHOP, or DCDF

The data for the effect of (R,S)-α-chlorohydrin and CHOP on rat sperm have already been published (Bone and Cooper, 2000) and are repeated here solely for comparison. Sperm suspensions from all species were diluted to 24 × 10^6/mL with temperature- and CO_2-equilibrated medium G. Defined volumes (rat, 800 μL; mouse and human, 550 μL) of sperm suspension were diluted in the same volume of medium G alone (control) or medium G containing 0–200 mM (R,S)-α-chlorohydrin, 0–20 mM CHOP, or 0–40 mM DCDF, and were incubated for 2 hours at 37°C in 5% CO_2. Addition of ACH, CHOP, and DCDF required lowering the NaCl content of medium G to maintain osmolality at 310 ± 5 mmol/kg. Final sperm concentrations were 12 × 10^6/mL.

Video recordings for computer-assisted sperm analysis (CASA) were made before washing the rodent cells (1 mL) through 5 mL of 5% Ficoll (Heraeus; 1000 g [rats], 2000 g [mice] for 7 minutes at 4°C), removing most of the supernatant solution to leave the pellet in 240 μL (rat), 51 μL (mouse), or 102 μL (human). Sonication buffer (100 mmol/L triethanolamine, 5 mmol/L ethylenediamine tetraacetic acid-Na_2, 1 mmol/L dithiothreitol pH 7.3) was added (rat, 460 μL; mouse, 99 μL; human, 198 μL) and the resulting suspension was sonicated three times for 7 seconds at the highest intensity with cooling on ice between sonications (3-mm tip, KLN-Ultraschallgenerator, Heppenheim, Germany; or 1.5-mm tip, Vibra-Cell-Sonicator, Sonics and Materials Inc, Danbury, Conn). The sperm concentration of the resulting sonicates was estimated by nephelometry (rats and mice) or hemocytometry (human). Finally, the activities of GAPDH, hexokinase (HK), and TPI were measured.

Measurement of Sperm Kinematics (CASA)

At the end of incubation a 4-minute video recording was made for motility estimation by CASA (Hamilton-Thorne IVOS; Beverly, Mass) using distinct settings for each species (Table). A relatively low frame rate was chosen to accentuate different swim paths (Yeung et al, 1992). The following parameters were measured: curvilinear velocity (VCL; time-averaged velocity of a sperm head along its actual curvilinear trajectory), straight line velocity (VSL; time-average velocity of a sperm head along the straight line between its first detected and last positions), and linearity (LIN; linearity of the curvilinear trajectory = VSL/VCL × 100). The percentage of motile cells (MOT) was estimated visually by counting a minimum of 200 motile and immotile sperm.

Parameter settings of the Hamilton-Thorne for analyzing spermatozoal kinematics

	Rat	Human	Mouse
HTM-IVOS Version	10.6 h	10.6 h	10.6 h
Frame rate, Hz	12.5	25	12.5
No. frames	30	25	30
Minimum contrast, gray level	30	60	30
Minimum size, pixel	20	4	6
Magnification	0.63	1.56	0.63
Minimum no. tracking points	10	13	16
VAP, μm/s	10–500	5–500	3–500
VCL, μm/s	15–500	—	—

Measurement of Enzyme Activity in Sperm

The glycolytic enzymes GAPDH (EC 1.2.1.12), HK (EC 2.7.1.1), and TPI (EC 5.3.1.1) in sperm sonicates were measured by spectrophotometry as described by Bergmeyer (1974) and Ford and Harrison (1981). Methods were modified for a 96-well plate format on 25-μL or 50-μL extracts. GAPDH and HK activities were assessed on undiluted sonicates, whereas TPI was measured after fivefold dilution of the sonicate. Assayed activities were linear with time and volume of extracts assayed (not shown).

Statistics

For each sample, kinematic parameters were expressed as the median of analyzed sperm tracks per animal. The data in the text and graphs are the mean values of each treatment group expressed as the percentage of control values. Control values are given in the Figure 2 legend. Comparison between doses of ACH, CHOP, and DCDF was made by two-way repeated measures analysis of variance and differences between species were established by Tukey's test. Differences were considered significantly different when $P < .05$.

Results

Effects of (R,S)-α-Chlorohydrin on Motility and Glycolytic Enzymes of Spermatozoa In Vitro

Spermatozoa of all the species investigated (rat, mouse, human) were susceptible to (R,S)-α-chlorohydrin. A change in motility, kinematic parameters and the activities of GAPDH and TPI were observed with increasing (R,S)-α-chlorohydrin concentrations. HK, as a control enzyme, was not effected (not shown). Spermatozoa of mouse were most sensitive to (R,S)-α-chlorohydrin with the most prominent decrease in kinematic parameters at 1 and 10 mmol/L and the strongest GAPDH and TPI inhibition at 0.1 to 10 mmol/L (Fig. 2, left panels). The degree of inhibition of kinematic parameters of rat sperm was more pronounced than that of human sperm, and the inhibition

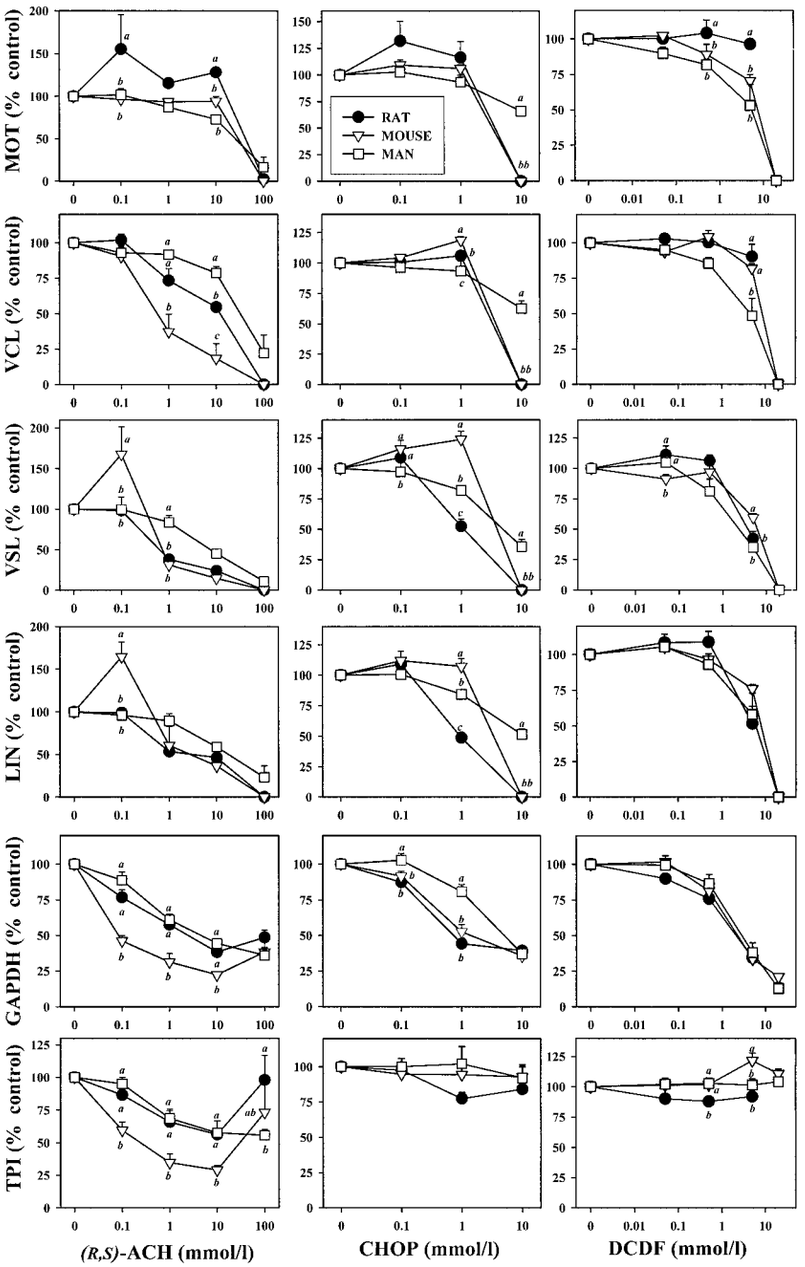

Figure 2. In vitro inhibition by α-chlorohydrin (left panels), 1-chloro-3-hydroxypropanone (middle panels), and 1,6-dichloro-1,6-dideoxyfructose (right panels) of sperm kinematics: **(a)** MOT, **(b)** VCL, **(c)** VSL, **(d)** linearity (LIN); and the activities of the glycolytic enzymes, **(e)** GAPDH and **(f)** TPI. For each compound and parameter, values are expressed as percentages of controls (mean ± SEM) for rats (●, n = 4 ACH, DCDF; n = 7, CHOP), mice (▽, n = 4 ACH, CHOP, DCDF, except 100 mmol/L, n = 2) and man (■, n = 4 ACH, CHOP; n = 5, DCDF, except 20 mmol/L, n = 3). At each dose, values bearing different superscripts are significantly different between species ($P < .05$). Control values in the absence of ACH in the rat were as follows: VSL, 58.1 ± 6.4; VCL, 98.9 ± 4.9 μm/s; LIN, 60.3 ± 2.6; MOT, 32.6% ± 4.9%; GAPDH, 7.32 ± 4.93; TPI, 32.3 ± 6.6 mU/10^6 sperm. In the mouse, values were as follows: VSL, 35.6 ± 1.6; VCL, 110.4 ± 1.3 μm/s; LIN, 36.0 ± 1.5; MOT, 69.1% ± 8.8%; GAPDH, 7.00 ± 0.92; TPI, 24.8 ± 2.5 mU/10^6 sperm. For the human, values were as follows: VSL, 45.6 ± 8.2; VCL, 69.8 ± 8.2 μm/s; LIN, 69.5 ± 4.3; MOT, 43.9% ± 10.1%; GAPDH, 8.67 ± 0.87; TPI, 26.9 ± 5.5 mU/10^6 sperm. Control values in the absence of CHOP in the rat were as follows: VSL, 50.4 ± 3.8; VCL, 86.5 ± 1.4 μm/s; LIN, 57.8 ± 4.2; MOT, 41.0% ± 5.7%; GAPDH, 6.70 ± 0.55; TPI, 53.3 ± 5.2 mU/10^6 sperm. In the mouse, values were as follows: VSL, 45.9 ± 2.9; VCL, 146.2 ± 3.9 μm/s; LIN, 33.1 ± 1.5; MOT, 46.1% ± 3.5%; GAPDH, 4.04 ± 0.57; TPI, 9.8 ± 2.6 mU/10^6 sperm. For the human, the values were as follows: VSL, 44.7 ± 4.7; VCL, 68.5 ± 3.3 μm/s; LIN, 74.0 ± 5.3; MOT, 34.8% ± 1.3%; GAPDH, 3.04 ± 0.43; TPI, 6.89 ± 0.79 mU/10^6 sperm. Control values in the absence of DCDF in the rat were as follows: VSL, 44.1 ± 4.5; VCL, 82.1 ± 2.1 μm/s; LIN, 55.0 ± 6.3; MOT, 52.2% ± 3.4%; GAPDH, 6.10 ± 0.36; TPI, 25.6 ± 3.3 mU/10^6 sperm. In the mouse, values were as follows: VSL, 55.4 ± 1.1; VCL, 147.2 ± 9.1 μm/s; LIN, 39.4 ± 1.7; MOT, 58.7% ± 4.0%; GAPDH, 4.35 ± 0.40; TPI, 14.0 ± 1.4 mU/10^6 sperm. For human, values were as follows: VSL, 49.7 ± 5.3; VCL, 78.2 ± 4.8 μm/s; LIN, 71.1 ± 5.9; MOT, 37.9% ± 3.5%; GAPDH, 7.17 ± 0.92; TPI, 12.4 ± 4.6 mU/10^6 sperm.

was significantly different from inhibition in human sperm for VSL at 1 mmol/L and VCL at 10 mmol/L. The extent of inhibition of GAPDH and TPI after 2 hours was similar for human and rat sperm.

The percentage of motile sperm remained unchanged up to a concentration of 10 mmol/L in all species, but 100 mmol/L led to a drastic reduction in percentage motility. In rat and mouse, the inhibition of sperm GAPDH at 10 mmol/L was partly reversed, and the inhibition of TPI was nearly completely reversed by 100 mmol/L, whereas in human sperm the level of inhibition was maintained. For murine sperm there was an unexpected but consistent stimulation of VSL and LIN of about 60% during 2 hours of incubation in 0.1 mM, but not at the higher doses, although no changes in VCL and percentage motility were apparent at this concentration, and no changes in enzyme activity were observed (Fig. 2, left panels).

Effects of CHOP on Motility and Glycolytic Enzymes of Spermatozoa In Vitro

Up to a concentration of 1 mmol/L there was no change in global motility of rat and mouse sperm (Fig. 2, middle panels). At 10 mmol/L, total immotility of rat and mouse sperm was achieved, whereas 65% of human sperm remained motile. Therefore, kinematic parameters of human sperm at 10 mmol/L CHOP were significantly higher than in sperm from the other species. A species-dependent variation of the effect of CHOP was found at a concentration of 1 mmol/L. The degree of reduction of VSL and LIN fell in the order of rat > human > mouse. The inhibition of GAPDH was similar in rats and mice, but was significantly lower in human sperm at 0.1 and 1 mmol/L. Moreover, in contrast to ACH, CHOP did not inhibit TPI activity, nor the activity of the control enzyme, HK (not shown).

Effects of DCDF on Motility and Glycolytic Enzymes of Spermatozoa In Vitro

As for ACH, DCDF decreased kinematic parameters and activities of GAPDH in all species in a dose-dependent manner, but the extent of inhibition of sperm from different species was similar with slightly stronger reductions in human sperm. Like CHOP, DCDF did not block TPI activity. HK activity was unaltered (not shown). DCDF at 20 mmol/L rendered mouse and human sperm immotile (Fig. 2, right panels).

Discussion

The present study was performed to examine whether the putative precursors of 3-chloro-lactaldehyde, ACH, CHOP, and DCDF, were able to inhibit the sperm glycolytic enzymes, GAPDH and TPI, in different species in vitro. The effect on sperm glycolysis was checked by releasing sperm into glucose-only-containing medium, estimating kinematic parameters by CASA, and evaluating enzyme activities. All 3 compounds tested inhibited sperm GAPDH and kinematic parameters in rats, mice, and humans, but there were differences regarding the lowest effective concentrations and the inhibition of TPI activity.

Despite the toxicity of high doses of (R,S)-α-chlorohydrin and its methylpentyl dioxolane, which prevented mating of mice, sperm recovered from their epididymides displayed reduced fertilizing capacity when used in vitro or when inseminated into the uterus (Tsunoda and Chang, 1976a, 1976b). Administration of (S)-3-amino-1-chloropropanol to male mice also lowers their fertilizing capacity in vitro, but not after insemination (Tsunoda and Chang, 1976b). These results suggest that mouse sperm are susceptible to the effects of (R,S)-α-chlorohydrin in vivo and thus should contain $NADP^+$-dependent glycerol dehydrogenase (GDH), the enzyme that converts (S)-α-chlorohydrin to (S)-3-chlorolactaldehyde (Stevenson and Jones, 1985). Indeed, Ford and Harrison (1983) demonstrated reduced GAPDH activity of both proximal and distal epididymal spermatozoa from mice fed high doses of (S)-α-chlorohydrin for 10 days. The present in vitro work confirms this supposition and demonstrates that murine sperm GAPDH is susceptible to inhibition by (S)-3-chlorolactaldehyde whether generated from ACH via GDH, from CHOP via TPI, or from DCDF via aldolase and TPI.

The stimulatory action of low concentrations of (R,S)α-chlorohydrin in vitro on murine sperm is currently inexplicable because no stimulation of glycolytic activity was demonstrable. A similar stimulation of motility was observed in rat sperm upon their immediate release from the epididymis into medium containing metronidazole (Cooper, 1992), an ornidazole analogue that lacks short-term antifertility action in rats (Cooper et al, 1997), and thus is also unlikely to affect sperm metabolism.

For boar sperm, coincubation with 10 mmol/L DCDF causes a drastic inhibition of their GAPDH activity, whereas the TPI activity remains unaffected (Jones and Morin, 1995) and aldolase (EC 4.1.2.7) is transiently inhibited by DCDF. These results indicate metabolism of DCDF to (S)-3-chlorolactaldehyde by porcine sperm in vitro. In the present study, similar in vitro effects of DCDF on reducing GAPDH activity, while sparing TPI activity, were demonstrated for sperm from rats, mice, and men. DCDF is therefore a promising agent for the evaluation of contraceptive potential in animal systems. Because DCDF contains 2 chlorine atoms, the action of aldolase should produce 2 potential inhibitory agents, (S)-3-chlorolactaldehyde and CHOP, of which the latter is further converted by TPI to (S)-3-chlorolactaldehyde. Thus,

1 mole of DCDF produces the equivalent of 2 moles of (R,S)-α-chlorohydrin, so that immotility was produced at 40 rather than 20 mmol/L.

The decrease in glycolytic enzyme activity after release of sperm from rats, mice, and humans into CHOP-containing medium parallels the decline in lactate and CO_2 production observed in boar sperm (Jones et al, 1986). Rodent epididymal sperm were more susceptible to inhibition than human ejaculated sperm with regards to both enzymes. As with DCDF, TPI activity was uninfluenced by CHOP, in contrast to porcine sperm (Jones, 1987; Jones and Cooney, 1987).

The differences in the inhibition of TPI by ACH, CHOP, and DCDF may be explained by the distinct metabolism of these compounds. (S)-ACH is metabolized to (S)-3-chlorolactaldehyde by an $NADP^+$-dependent GDH. The product will subsequently bind to the active center of TPI and be converted to CHOP, which is likely to be the main product in the equilibrium. As the TPI assay was performed with glyceraldehyde 3-phosphate (GAP) as substrate, (S)-3-chlorolactaldehyde formed from the exogenous ACH would be able to compete with GAP via binding to the active center of TPI and inhibiting its activity. If the steady state of TPI favors CHOP as it does dihydroxyacetone phosphate (Barman, 1969), less CHOP would be converted to 3-chlorolactaldehyde, although clearly a sufficient amount was formed to block the activity of GAPDH. GAP would also compete more easily with CHOP if it does not bind as tightly to the active center of TPI as does (S)-3-chlorolactaldehyde. This situation may be similar for DCDF, in which (in the boar) at least 50% is converted to CHOP (Jones and Morin, 1995).

Previous in vitro studies on human spermatozoa have indicated that only high concentrations of (R,S)-α-chlorohydrin are capable of inhibiting sperm motility and metabolism (0.3 M racemic mixture; Homonnai et al, 1975). The conclusion drawn was that human sperm were relatively refractory to the effects of (R,S)-α-chlorohydrin, which would therefore not be a useful contraceptive agent for men. By stark contrast, the results of the present study indicate that GAPDH and TPI activities of human ejaculated sperm are no less sensitive to the effects of redistilled (R,S)-α-chlorohydrin in vitro than are rat epididymal sperm. This difference may well reflect the purity of the chlorohydrin preparations in the older studies, which if undistilled, contain many impurities (Jones and Cooper, 1999). Furthermore, human spermatozoa were also susceptible to inhibition by other chlorinated compounds and equally susceptible as rat and mouse sperm to DCDF and slightly less susceptible to CHOP. Ford et al (1979) demonstrated >80% inhibition of human sperm metabolism by 50 mmol/L (R,S)-ACH and greater inhibition by the (S)-enantiomer. Because the racemic mixture was used here, the concentration of ''effective'' (S)-enantiomer is half that indicated, reducing the difference in apparent efficacy between the chlorinated compounds tested.

These in vitro results auger well for these agents to be considered for post-testicular activity in men. However, although murine sperm are sensitive to the effects of (R,S)-α-chlorohydrin in vitro, males remain fertile after being fed the compound (Samojlik and Chang, 1970), an observation most likely explained by the poor penetration of ACH to the epididymis, as proposed by Crabo and Apelgren (1972), because the sperm are inhibited by ACH in vitro. Distribution studies on nonhuman primates are needed to determine whether this inhibition could become a basis for contraception in man.

Acknowledgments

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