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Short Paper

Competition of zinc, cadmium and calcium for binding sites in sperm of trout and carp

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Summary

Heavy metal pollutions of aquatic ecosystems have already raised many concerns on aquatic organisms'health and survival especially on their sperm. The effects of a single metal on sperm may be totally different from cocktail of several metals because of their interactions, so the effects of zinc, cadmium and calcium on the trout and carp sperms and their competition have been examined by incubation of different concentrations of cold heavy metals with a radioactive competing metal. The results showed that radioactive ^{65}Zn and ^{109}Cd were not displaced from the trout sperm by calcium even at 3000 ppm concentration but radioactive cadmium was displaced equally well by cadmium or zinc, with a decrease in pellet activity to 38 and 37% of control values (0 ppm concentration) at 3000 ppm concentration, respectively in trout. Calcium was replaced by non-radioactive and radioactive zinc and cadmium as well as by calcium. The findings confirmed that at least part of heavy metals toxicity (especially cadmium) on fish sperm could be due to displacement of essential zinc and calcium. Both trout and carp sperms showed a similar pattern.

Key words: Sperm, Zinc, Cadmium, Pollution, Competition

Introduction

Trace elements occur in much higher concentrations in seminal plasma than in the other body fluids of animals (Huang *et al*., 2000). This high concentration of some trace elements in seminal plasma, may be related to an essential role on sperm activity (Abou Shakra *et al*., 1989). Among trace elements, zinc is essential for reproduction in many animals. Spermatozoa and seminal fluid from several mammalian species are rich in zinc (Wong *et al*., 2001), which derive, at least in part, from prostatic secretion (Telisman *et al*., 2000) and it has been suggested that, in mammals, this element has an important role in the male reproductive system (Behne *et al*., 1988) and may be related to sperm motility (Telisman *et al*., 2000). There is some evidence that zinc is necessary for normal growth and sexual maturation (Gaur *et al*., 2000), spermatogenesis (Wong *et al*., 2001), sperm motility (Wong *et al*., 2001), stabilization of sperm chromatin (Bench *et al*., 1999), membrane activity during fertilization (Ahluwalia *et al*., 1991), normal functioning of the hypothalamic-pituitary-gonadal axis (Millar *et al*., 1960) and is involved in regulation of sperm motility in the sea urchin via intracellular pH (Telisman *et al*., 2000).

Cadmium is extremely toxic to spermatozoa even at concentrations as low as 10^{-6} M (0.11 ppm) (Wade *et al*., 2002) and is able to induce genotoxic damage in both somatic and germ cells (Mukherjee *et al*., 1988). Cadmium treatment also affected testis and epididymus weight, sperm concentration and serum FSH in old rats (Bellas *et al*., 2001).

It has been suggested that the harmful effect of cadmium may be due to interaction of cadmium with endogenous zinc (Battersby *et al*., 1982a) which may jeopardize sperm chromatin stability by reducing chromatin zinc content (Rosenborg *et al*., 1990). Since in mature spermatozoa, the zinc ion is presumed to stabilize sperm chromatin through a reversible binding to its thiol groups (Fabiani *et al*., 1995). It may also act

through inactivation of glyceraldehyde phosphate dehydrogenase, an important enzyme that functions as part of the spermatozoal glycolytic process in the production of energy-rich ATP (respiration process) (Earnshaw *et al*., 1986). This inhibition of respiration process has been confirmed in sea urchin spermatozoa exposed to zinc and cadmium, to which addition of ATP retrieved flagella motility (Yoshida *et al*., 1994).

Zinc and cadmium have similar chemical properties (Gatewood *et al*., 1990) and both decrease the quantity of glucose utilization and glucose oxidization by spermatozoa (Holland and White, 1988) and may compete for binding sites on proteins or on metallothioneins (Gatewood *et al*., 1990). Cadmium ions are also extremely potent at blocking the response to Ca^{2+}, completely eliminating the curvature reversal of sperm (Kanous *et al*., 1993) and non-competitively inhibit Ca^{2+} transport (Visser *et al*., 1993). It has been suggested that the effects of metal ions such as calcium and zinc on sperm motility may be mediated through a common cation-binding site on the adenyl cyclase (Magnus *et al*., 1990). By analysing DNA sequence in ATPase N-terminal, it has been suggested that zinc and cadmium have a common binding and transportation site (Heinz *et al*., 2005, Liu *et al*., 2005). The effects of zinc, cadmium and calcium on sperm and their competition have been examined by using the trout sperm as experimental model.

Materials and Methods

Fish

Six male trout, (1057 ± 310 (SD) g; GSI 1.2-3.4) were purchased from a local trout farm and kept in 1000 l tanks with circulating freshwater at 10°C. Milt was collected by gently stripping the abdominal walls. Carp's milt was collected 24 hrs after hypophysation from six fish (1-2 kg) held in another local farm and transported on ice to Sheffield, England).

Incubations

Fifty µl of milt from individual fish of each species was incubated in plastic tubes for 24 hrs, with 0.925 kBq ^{65}Zn (Sp. Act. 2.41 GBq/mmol, New England Nuclear) or ^{109}Cd (Sp. Act. 4.03 GBq/mmol, New England Nuclear) in 2 ml sperm extender medium containing 0, 1, 3, 10, 30, 100, 300, 1000 and 3000 ppm zinc (0-46 mM), cadmium (0-27 mM), or calcium (0-75 mM). The sperm extender medium used for carp was: 5.52 g/l NaCl, 2 g/l KCl, 2.42 g/l tris-HCl, 3.75 g/l glycine in distilled water and pH = 7.5 (Kime *et al*., 1996). The medium for trout was as described by Miura *et al*., (1992). Incubation temperatures were 20 and 10°C for carp and trout, respectively.

Fifty µl of trout milt was also incubated for 24 hrs at 10°C with 0.37 kBq ^{45}Ca (Sp. Act. 18.9 MBq/mmol, Amersham International, UK) in 2 ml trout extender medium containing the same concentrations of zinc, cadmium or calcium as mentioned above.

At the completion of incubation, tubes were centrifuged (2616 rpm, 15 min), the supernatant removed and ^{65}Zn and ^{109}Cd counted directly in a γ-counter (Packard Cobra 5002). Instrument setting for ^{59}Fe and ^{125}I were used to count radioactivity of ^{65}Zn and ^{109}Cd, respectively; since these isotypes have similar energy levels. Tissue containing ^{45}Ca was solubilized in 300 µl NCS Tissue Solubilizer (Amersham International, UK) and 50 µl of the solution added to 20 ml scintillation fluid (Emulsifier scintillator plus, Packard) for counting (Packard Model 1600 TR). The percent activity of radioactive metals calculated by dividing the counted activity (disintegrations per minute = dpm) in treatment incubation (B) by the activity counted in control incubation (B_0).

Statistical analysis

Data were analysed using the paired-sample t-test (SPSS 6 for Windows; SPSS Inc.) for comparison between the mean (six fish sperm incubations) activity of each concentration counted with 0 ppm concentration (control) in incubation of sperm with non-radioactive metals.

Results

Trout sperm incubations

Displacement of ^{65}Zn by zinc and cadmium: the activity of ^{65}Zn in the pellet decreased from 71.1 and 90.5% to 29.6 and

54.2% of the control value (0 ppm of non-radioactive) in the presence of 3000 ppm non-radioactive zinc or cadmium, respectively; so ^{65}Zn were as well displaced by non-radioactive zinc and cadmium in trout sperm (Fig. 1, Table 1).

Displacement of ^{109}Cd by zinc and cadmium: radioactive cadmium was displaced by cadmium or zinc, with a decrease in the pellet activity from 74.9 and 83.4% to 37 and 35.2% of the control values (0 ppm concentrations of cold zinc and cadmium) at 3000 ppm concentration, respectively (Fig. 1).

Displacement of ^{45}Ca by calcium, zinc or cadmium: the activity in the pellet decreased from 86.9, 95.9 and 95.9% to 30, 60.4 and 58.5% of the control value (0 ppm concentrations of non-radioactive) at 3000 ppm calcium, zinc and cadmium, respectively; calcium could be replaced by non-radioactive zinc and cadmium as well as by calcium (Fig. 1).

Carp sperm incubations

Displacement of ^{65}Zn by zinc or cadmium: the activity of ^{65}Zn in the pellet decreased from 89.9 and 70.3% to 52.1 and 28.2% of the control value (0 ppm of non-radioactive) in the presence of 3000 ppm non-radioactive zinc or cadmium, respectively; so ^{65}Zn were displaced by non-radioactive zinc and cadmium (Fig. 2, Table 2).

Displacement of ^{109}Cd by zinc or cadmium: radioactive cadmium was displaced by cadmium or zinc, with a decrease in the pellet activity from 84.7 and 75.2% to 38.1 and 37.2% of the control values (0 ppm concentrations of cold zinc and cadmium) at 3000 ppm concentration, respectively. Radioactive cadmium was not displaced by calcium (Fig. 2, Table 2).

Discussion

The concentrations of heavy metals used for sperm incubations were in accord with the previous studies using the same conditions to study sperm motility in fish (Kime *et al*., 1996; Rurangwa *et al*., 1998). Sperm may be in contact with metal pollutants inside the testis due to bioaccumulation process and it has already been shown the same concentrations of metals may be available to sperm there (Ebrahimi, 2004). Usually, sperm may be exposed to such concentrations (used here) due to chronic or subchronic water contaminations during its development and before they were released out (Kime *et al*., 1996; Rurangwa *et al*., 1998).

The importance of not considering the effects of individual pollutants in isolation has been shown so far (Kime *et al*., 1996) since the effect of cocktail of toxicants in polluted environments may not simply be the sum of individual effects. The interaction of zinc and cadmium was examined by

Table 1: The percentage of activity counted in incubations of trout sperm with radioactive metals (^{65}Zn, ^{109}Cd or ^{45}Ca) in the presence of different concentrations of (0-3000 ppm, non-radioactive) zinc, cadmium or calcium (B/B_0 = dpm bound / dpm bound with radiolabel alone)

	1	3	10	30	100	300	1000	3000
^{65}Zn								
Ca	89.6	89.7	91.1	90.3	92.2	90.5	91.1	89.9
Zn	89.6	87.6	78.6	72.6	68.4	63.1	58.9	52.1
Cd	70.3	54.6	42.1	39.5	37.1	36.2	35.4	28.2
^{109}Cd								
Ca	96.1	95.6	95.9	95.2	96.2	94.2	92.3	89.2
Zn	75.2	75.1	58.9	56.2	48.1	46.2	43.8	37.2
Cd	84.9	66.9	65.8	58.1	52.8	46.5	42.4	38.1
^{45}Ca								
Ca	86.9	85.8	72.4	67.3	52.6	46.4	35.4	30
Zn	95.9	94.8	89.7	81.9	75.8	71.4	69.7	60.4
Cd	95.9	94.5	89.5	81	74.1	69.5	61.4	58.5

Fig. 1: The effect of 0-3000 ppm calcium (❍), cadmium (■) and zinc (□) on binding of ^{65}Zn, ^{109}Cd or ^{45}Ca on trout sperm. B/B_o = dpm bound/dpm bound with radiolabel alone (*indicates a significant difference ($P<0.05$) in displacement between zinc and cadmium (for ^{65}Zn and ^{109}Cd, or between calcium and zinc, or calcium and cadmium (for ^{45}Ca) at the same concentration)

Fig. 2: The effect of 0-3000 ppm cadmium (■) and zinc (□) on binding of ^{65}Zn or ^{109}Cd on carp sperm. B/B_o = dpm bound/dpm bound with radiolabel alone (*indicates a significant difference ($P<0.05$) in displacement between zinc and cadmium at the same concentration)

Table 2: The percentage of activity counted in incubations of carp sperm with radioactive ^{65}Zn and ^{109}Cd in the presence of different concentrations (0-3000 ppm) of non-radioactive zinc and cadmium (B/B_o = dpm bound / dpm bound with radiolabel alone)

	1	3	10	30	100	300	1000	3000
^{65}Zn								
Zn	71.1	53.2	46.5	40.3	38.4	36.4	34.2	29.6
Cd	90.5	78.5	76.7	72.1	68.1	58.4	56.4	54.2
^{109}Cd								
Zn	83.4	69.4	67.9	56.2	50.4	49.5	39.3	35.2
Cd	74.9	57.6	52.6	48.9	46.3	44.7	41.6	37

measuring displacement of radioactive cadmium and zinc by the non-radioactive

metals in in vitro incubations of sperm from two teleost species. The results in the present study showed that ^{65}Zn can be displaced by cadmium at higher concen-trations and it supports the previous finding suggested that cadmium may be toxic due to displacement of essential zinc from its binding sites (Kime *et al*., 2001). Battersby *et al*., (1982b) have also suggested that the harmful effects of cadmium may be due to displacement of the endogenous zinc, which stabilizes sperm chromatin by binding reversibly to the thiol groups of mature spermatozoa (Rousseaux and Rousseaux Prevost, 1995). Such suggestions are also supported since cadmium treated rat sperm showed a significant reduction in nuclear zinc (Battersby *et al*., 1982a).

It has already been shown that the toxicity of cadmium was decreased in the presence of zinc (Kime *et al*., 1996). The present results clearly show that in sperm of carp and trout, zinc and cadmium can compete for binding sites since they have similar chemical properties (Gatewood *et al*., 1990) and confirm the previous suggestion (Kime and Singh, 1996) that the protective effect of zinc against cadmium toxicity is attributable to displacement of cadmium. The finding in the present study is in agreement with the protective effect of zinc against testicular injuries by lead + cadmium exposure in rats (Saxena *et al*., 1989) and increasing sperm flagellar beat in cadmium exposure (Battersby *et al*., 1982b). It has already been shown that the progressive motility of trout sperm was decreased after exposure to 100 ppm cadmium or 2000 ppm zinc in extender for 24 hrs and cadmium toxicity on sperm motility was significantly decreased in the presence of an equal amount of zinc (Kime *et al*., 1996).

Calcium regulates several properties of sperm including initiation and maintenance of sperm motility in mammalian sperm (Tash *et al*., 1988). Alteration of calcium balance could result in disruption of sperm motility. As apparently showed in this study zinc and cadmium compete with calcium in binding to sperm and at high concentration of these heavy metals they can remove essential calcium from its binding site. At least part of the zinc and cadmium spermiotoxicity shown could be due to replacement of calcium or to disturbing the intra and extracellular calcium balance. Alteration of this balance has been shown to disturb spermatozoa motility in rainbow trout (Okuno and Morisawa, 1989). The extreme potency of cadmium in blocking the response of sperm to Ca^{2+} has already been shown (Lindemann *et al*., 1991), since cadmium can non-competitively inhibit Ca^{2+} transport (Visser *et al*., 1993). Previous studies are in agreement with the findings in the present study that the toxic effect of cadmium in sperm may be due to displacement of either essential zinc or calcium.

The results clearly show that the heavy metals toxicity may be caused by displacement of essential metals (zinc and calcium) from binding sites in sperm. No differences were seen between the two fish species studied here (trout and carp), the patterns of heavy metals toxicity were similar. Therefore, such competition for binding sites may be applicable to heavy metals toxicity in other aquatic organisms.

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