Effects of some non steroidal anti-inflammatory drugs on rat sperm hyperactivation *in vitro*

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Abstract

The aim of the study was to investigate the effect of 3 primarily COX-1 inhibiting non-steroidal anti-inflammatory drugs (aspirin, ibuprofen or indomethacin) on rat sperm hyperactivation *in vitro*. Rat cauda epididymal sperm were continuously incubated with different concentrations of drugs (aspirin: 0.87, 1.7 or 3.4 mmol; ibuprofen; 0.75, 1.5 or 3.0 mmol; and indomethacin; 0.43, 0.87, or 1.7 mmol) in a modified BWW medium at 37°C for 4 h. At the end of 4 h, the number of sperms exhibiting hyperactivated motility (characterized by vigorus tail movements, marked lateral excursion of head and following a nonlinear swimming trajectory such as figure eight or circling) and total immobility were counted and expressed as a %. The results showed that all the 3 drugs significantly ($p<0.05$-0.01) inhibited hyperactivated sperm motility in a dose-related fashion. Further, all three concentrations of ibuprofen and indomethacin, and the high concentration of aspirin caused an elevation in the number of immotile sperm. It is concluded that moderate concentrations of COX-1 inhibiting non-steroidal anti-inflammatory drugs can impair rat hyperactivated sperm motility *in vitro*.

Key words: ibuprofen; aspirin; indomethacin ; NSAIDs; COX-1 inhibitors; ratsperm hyperactivation

Introduction

Hyperactivation of sperm, which is generally recognized to be a component of capacitation (1,2,3), is now regarded as an absolutely essential prerequisite for penetration through the cumulus mass and zona pellucida (4). A variety of agents in mild to moderate concentrations are known to inhibit sperm hyperactivation in vitro. These include amino acids (5,6), neurotransmitters (7), calcium channel blockers (1), calmodulin antagonists (1) and analgesics (8). However, cyclooxygenase (COX) inhibitors have not been tested for their effects on sperm hyperactivation although prostaglandins (9) and prostonoid receptors (9,10) are claimed to be present in sperm. In this study, we investigated the impact of some selected COX-1 inhibitors on rat sperm *in vitro*.

Materials and Methods

Animals

Adult crossbred male albino rats (240-260 g) from our own colony in the Department were used. They were kept under standardised animal house conditions (temperature: 28-30°C; photoperiod: approximately 12 h light / 12 h dark; relative humidity: 50 – 55 %) with free access to pelleted food (Vet House Pvt. Ltd., Makola, Sri Lanka) and tap water.

Drugs

All the drugs used were purchased from Sigma Chemical Company, ST. Louis, MO USA.

Effect on hyperactivated sperm motility

Hyperactivated motility was assessed as described by Yanagamachi [11] and White *et al* [12]. Briefly, male rats were anaesthetised using ether and cauda epididymides were exposed and a distal tubule was punctured and spermatozoa were extruded into a modified Biggers Whitten Whittingham (BWW) medium (11). Sperm concentration was adjusted to $5'10^6$/ml; 200ml of BWW was placed at the center of a glass petri, dish and covered with liquid paraffin oil (BDH Chemicals Ltd., Poole, UK). Hundred microlitre of sperm suspension was then added to the BWW drop in the petri dish and mixed

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well. Then, 100ml of different concentrations (selected on the basis of their relative potencies as COX-1 inhibitors[13].) of either aspirin {6.8mmol (n=51), 3.4mmol (n=49), 1.7mmol (n=50)} or ibuprofen {6.0mmol (n=54), 3.0mmol (n=53), 1.5mmol (n=52)} or indomethacin {3.4mmol (n=46), 1.7mmol (n=44) or 0.86mmol (n=42)} or BWW (n=188) was added to the sperm suspension in the petri dish. These were incubated at 37°C for 4 h in 5%$C0_2$ in air under relative humidity of 95%. Nine microlitre of each of these sperm suspensions was then transferred on to a warm glass slide at (37°C) and was examined using a phase-contrast microscope (Olympus Optical Co. Tokyo, Japan) at 100 magnification and the number of hyperactivated sperms, and totally immotile sperms were counted (at least 100 spermatozoa were counted for each separate determination with aid of an eyepiece graticule). A spermatozoon was considered to be hyperactivated if it followed a nonlinear swimming trajectory such as figure eight or circling with vigrous tail movements and marked lateral excursions of head (11 12).

Statistical Analysis

The results are expressed as means ± SEM. Statistical comparisons were made using either Mann-Whitney U-test, or ANOVA followed by Turkey's post hoc test as appropriate. Significance was taken at $P < 0.05$.

Results

Over an incubation period of 4 h, in a modified BWW medium, with no added drugs 72-86% of caudal spermatozoa expressed typical hyperactivted motility (Figures 1-3). Addition of all the 3 drugs significantly ($P < 0.05$ to $P < 0.01$) impaired the number of sperms exhibiting hyperactivated motility in a dose-related manner (Figures 1-3): aspirin ($r^2=0.91$, $P<0.01$), ibuprofen ($r^2=0.82$, $P < 0.05$); and indomethacin ($r^2=0.99$, $P < 0.01$). The respective EC_{50} values for aspirin ibuprofen and indomethacin were 6.2, 3.7 and 0.4 mmol. Furthermore, with the addition of drugs many sperms become totally immobilized. (Table 1). Compared to control, ibuprofen and indomethacin significantly increased the number of immotile sperms at all 3 concentrations tested ($P < 0.05$ to 0.01) whilst aspirin had this effect only at the highest concentration.

Discussion

In this study, three widely used relatively cheap non-steroidal anti-inflammatory drugs (NSAIDs) (13) were used. All these three NSAIDs, despite belonging to different chemical classes (14) are primarily (COX-1) inhibitors (13).

The results showed that these 3 drugs induced antihyperactive and sperm immobilization effects against rat sperm *in vitro*. The precise mechanisms of action leading to the observed responses in sperm function remain unclear. Nevertheless, this is a novel, and an important finding: it shows that these drugs (at least, in moderate concentrations) are toxic to sperm. The maximum sperm immobilization effect with all the 3 drugs, was evident only at their highest concentrations. At the lowest concentration, only ibuprofen had immobilization effect. In contrast, a remarkable reduction on sperm hyperactivation was evident even at the lowest dose with all 3 drugs. Thus, the spermiostatic action is likely to be due to a non-specific effect such as plasma membrane perturbation (15) or toxicity (16). However, local application of aspirin (17) or indomethacin (18) in moderately high concentrations to the cauda epididymis of rats via silastic formulations has been shown to be nontoxic. Hence, a strong possibility exists that the immobilized sperm observed in this study were indeed viable. It is well recognized that nigrosin eosin stain cannot be used to test the viability of rat sperm. The antihyperactive effect of these 3 drugs was produced at relatively low concentrations (EC_{50} between 0.4-6.2 mM) and was concentration-dependant. Collectively, these findings and reported mode of action of these drugs(13,14)suggest that the antihyperactivation may be mediated by a selective action: most likely, sperm COX-1 inhibition. However, we have not determined the sperm prostaglandin level and used concentrations that usually exceed those required to inhibit COX-1 activitiy.

Very little is known of the biochemical pathways regulating expressions of hyperactivated motility (1,3). A substantial elevation of intracellular Ca^{++} is required to initiate hyperactivation (1,3,19). Both voltage sensitive L-type calcium channel blockers

such as dihydropyridines (1) and phenylalkylamines (1), and inorganic calcium channel blockers, or Ni^{++} (1,3) inhibit hyperactivation. However, NSAIDs do not possess calcium channel blocking activity (13,14) and therefore such a mode of action is unlikely. cAMP rise is implicated with expressed hyperactivated motility (1,3). In view of the reported mode of action of NSAIDs (13,14) , a cAMP mediated mechanism also seems unlikely to be operating here.

Aspirin can uncouple oxidative phosphorylation in addition to its COX inhibiting activity (13,14). This mode of action can also contribute for the aspirin induced impairment of sperm hyperactivation. Recently, it has been shown that ibuprofen and indomethacin impair nitric oxide (20) which has been implicated with sperm hyperactivation(21). Thus, with these two drugs this may have acted as an auxiliary mechanism to inhibit hyperactivation.

In conclusion, this study, for the first time, has shown that aspirin, ibuprofen, and indomethacin inhibit expression of rat sperm hyperactivation *in vitro.*

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Table 1 Sperm immobilizotion activity *in vitro* of different concentration of aspirin, indomethacin and ibuprofen (mean±SEM) $^{b}P<0.05$ and $^{c}P<0.01$, compared with controls (ANOVA followed by Tukey' post hoc test)

Drug	Concentrations (mmol)	% immotile sperm
Aspirin	0.0 (control)	14.8±2.2
	0.87	15.91±3.1
	1.7	17.6±3.7
	3.4	29.2±4.7^{b}
Indomethacin	0.0 (control)	21.3±3.8
	0.43	38.3±3.0^{c}
	0.87	44.8±3.7^{c}
	1.7	54.8±3.0^{c}
Ibuprofen	0.0 (control)	18.7±2.4
	0.75	45.6±2.3^{c}
	1.5	71.8±6.4^{c}
	3.0	89.2±3.5^{c}

Figure 1. Effects of aspirin (0.87, 1.7, or 3.4mmol) on hyperactivated sperm motility (at 4h of incubation) of rat *in vitro* (mean±SEM; n=150, $^{b}P<0.05$ compared with control, Mann-Whitney U test)

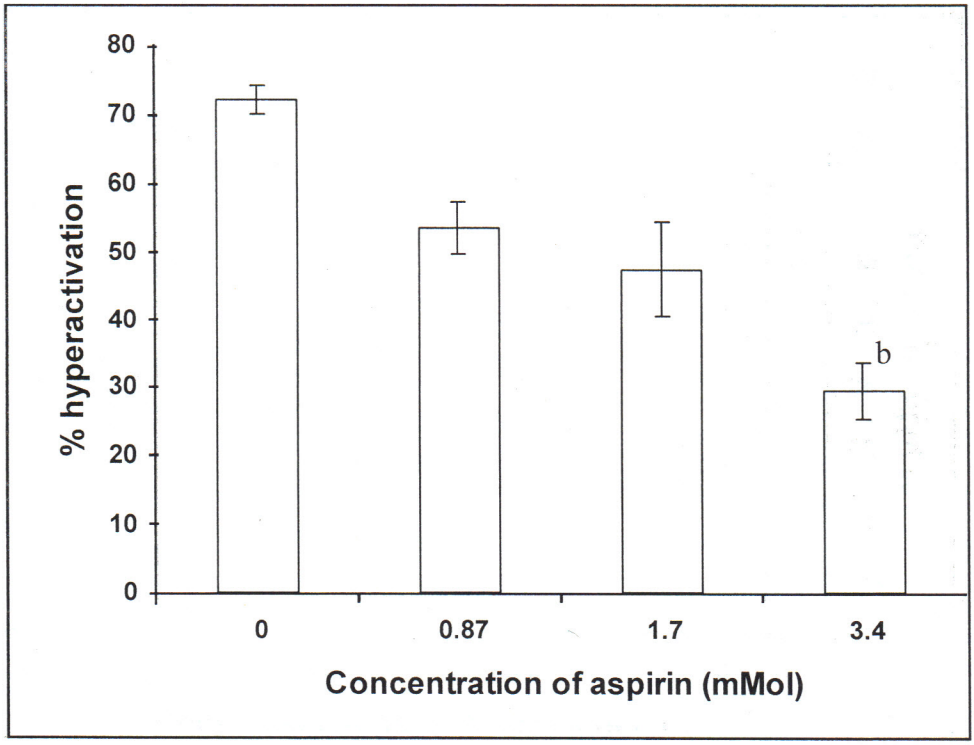

Figure 2. Effects of indomethacin (0.43,0.87 or 1.7mmol) on hyper activated sperm motility (at 4h incubation) of rat *in vitro* (mean ±SEM; n= 132, $^{c}P<0.01$: compared with control; Mann-Whiteny U-test)

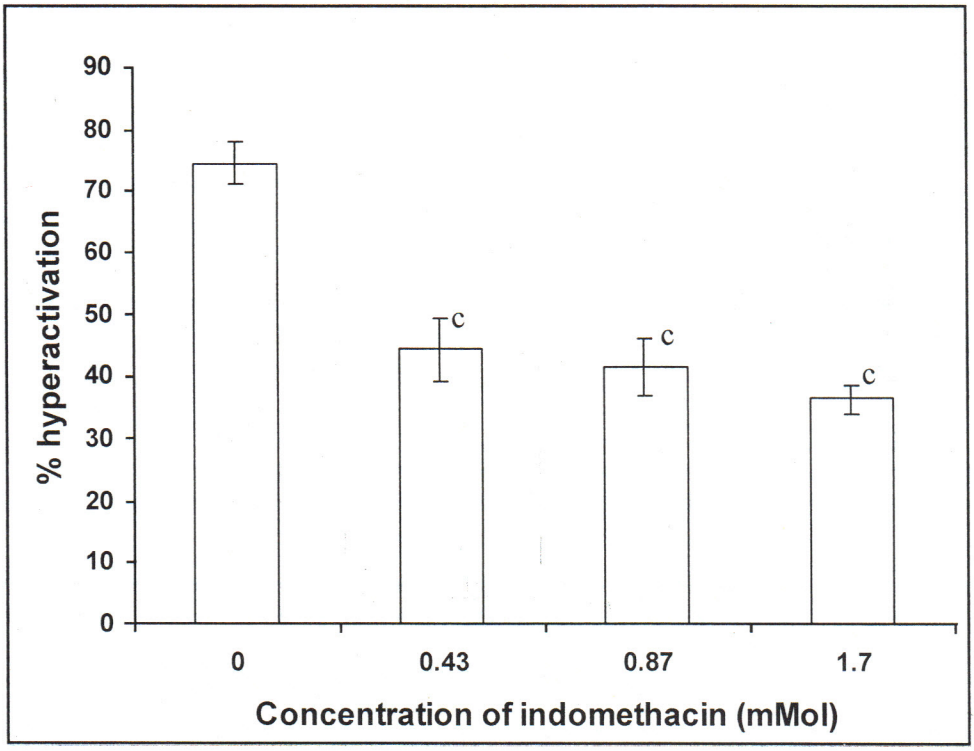

Figure 3. Effects of ibuprofen (0.75, 1.5 or 3.0mmol) on hyper activated sperm motility (at 4h of incubation) of rat *in vitro*(means±SEM;n=159,'*P*<0.01:compared with control; Mann-Whitney U-test)