EFFECT OF COX-2 INHIBITOR, ROFECOXIB, ON EPIDIDYMAL SPERM CHARACTERS AND ACCESSORY SEX GLANDS IN ADULT MALE RATES

F. K. Tawfeek, and S. M. Ahmed

Department of physiology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

ABSTRACT

The effect of long term administration of cox-2 inhibitor (Rofecoxib) on epididymal sperm characters (male fertility) was investigated in adult male rats by two separate experiments. Rofecoxib was administered orally at two doses 0.35 mg/Kg B.W., and 0.71 mg/Kg B.W. Animals received 0.35mg/Kg B.W. rofecoxib showed a significant increase in weights of head, body of epididymis, percentage of dead sperms and sperm abnormalities with a significant decrease in sperm count in the epididymal head and the percentage of live sperms. On the other hand, rofecoxib at a dose 0.71 mg/Kg B.W. caused a significant decrease in the percentage of live sperms accompanied with a significant increase in the percentage of dead sperms and sperm abnormalities, and the diameter of seminiferous tubules. It is concluded that the administration of rofecoxib was associated with an unexpected incidence of adverse effect on male rat fertility. However, the future might not look quite as satisfying as at first imagined, because it has become apparent that cox-2 inhibitors dose not simply have a significant role in pain and inflammation it might also work disadvantageously.

تأثير مثبط السايكلواوكسيجينيز-٢ (الروفيكوكسيب) على خصائص النطف والغدد الجنسية اللاحقة في ذكورالجرذان البالغة

فدوى خالد توفيق و سهى محمود احمد

فرع الأدوية والسموم والفسلجة والكيمياء الحياتية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تم دراسة تأثير مثبط السايكلواوكسيجينيز-٢ (الروفيكوكسيب)على خصائص نطف البـربخ (خـصوبة الـذكور) فـي ذكـور الجـرذان البالغـة بتجـربتين منفـصلتين. تـم أعطـاء الروفيكوكسيب بجرعتين ٠.٣٥ ملغم/كغم مـن وزن الجـسم و ٠.٧١ ملغـم/كغم مـن وزن الجسم عن طريق الفم لمدة ٦٠ يوما. اظهرت الحيوانـات المعاملـة بجرعـة ٠.٣٥ ملغم/كغم ارتفاعا معنويا في وزن رأس وجسم البربخ وزيادة النسبة المئوية للنطف الميتـة والتشوهات النطفية مع انخفاض معنوي في عدد نطف رأس البربخ مقارنـة مـع مجموعـة السيطرة. ام تلك المعاملة بجرعة و ٠.٧١ ملغم/كغم، فقد سببت انخفاضا معنويا في النسبة المئوية للنطف الحيـة مترافقـة مـع زيـادة معنويـة فـي النـسبة المئويـة للنطـف الميتـة والتـشوهات

النطفيــة ومعــدل اقطــار الانيبيبــات المنويــة. ويــستنتج مــن الدراســة ان المعاملــة بالروفيكوكسيب لمـدة ٦٠ يومـا عن طريق الفم قد اظهرت تأثيراتا" جانبيـة وغير متوقعـة على الخصوبة في الذكور.

INTRODUCTION

The various inflammatory mediators, prostaglandins, thromboxanes and leucotrienesare derived from phospholipids in the cell membrane via the cyclo-oxygenase (Cox) and lipooxygenase pathways (1). Prostaglandins found to be substantially involved in bringing about and maintaining inflammatory processes by increasing vascular permeability and amplifying the effects of other inflammatory mediaters such as kinins, serotonin and histamine (2). PG synthase catalyzes two separate reactions,the first being the cyclo-oxygenase function, which is the addition of molecular oxygen to arachidonic acid to form unstable PGG_2 (3). And the second, the future conversion of PGG_2 to the more stable PGH_2 by a peroxidase function. Hence, this cyclo-oxygenase enzyme performs the critical initial reaction in the arachidonic metabolic cascade leading to the formation of prostaglandins and prostacyclin (3). Cyclo-oxygenase exists in two forms Cox-1 and Cox-2.

Cox-1 can be detected in most tissues and typically expressed at constant levels throughout cell cycle, Cox-2 is undetectable in most mammalian tissues, but its expression can be rapidly induced in cells involved in inflammation. Therefore, Cox-1 has become known as the constitutive isoform and the Cox-2 as the inducible one, Cox-2 is constitutively expressed in brain, testes, tracheal epithelia and kidney (4). The predominant constitutive nature of Cox-1 together with the observations that expression of Cox-2 can be up regulated by inflammatory stimuli and the prostanoids are produced by Cox -2 in much larger amounts compared with Cox-1 has led to the hypothesis of the existence of (good) versus (bad) prostaglandins. According to this hypothesis, Cox-1 generates good prostaglandins for physiological (house keeping) functions, while Cox-2 forms the bad prostaglandins involved in inflammatory reactions and responsible for inflammatory sings (5). As a direct consequence, the specific inhibition of Cox-2 is expected to cause significant anti-inflammatory relief without interfering with prostaglandin mediated physiological processes, especially GI and renal function (6). Traditional non-steroidal anti-inflammatory drugs (NSAID) constitute one of the largest groups of pharmaceuticals. The most common uses of NSAID are to reduce pain, inflammation and fever (6). Other function of NSAID are still being investigated, such as antioncogenic, delayed labor, Alzheimer, breast cancer prevention effects (7,8). Since the increase in prostaglandins attributes to the inflammatory response, NSAID reduce inflammation and pain by inhibiting the cyclo-oxygenase activities (8). Cox-2 inhibitors constitute a new group of NSAID which at recommended doses, block prostaglandin production by cyclo-oxygenase-2, but not by cyclo-oxygenase-1 (9). Rofecoxib (vioxx)®, described chemically as 4-{4-(methyl sulphonyl) phenyl 3-3phenyl-2(5H)-furanone}, (10), is anew generation of nonsteroidal anti-inflammatory agent that exhibits promising anti-inflammatory, analgesic, antipyretic activity. It selectively inhibits Cox-2 isoenzyme in a dose dependent manner in man. No significant inhibition of Cox-1 is observed with rofecoxib up to doses of 1000mg (11). Various experimental models and clinical studies have demonstrated rofecoxib to be

superior, or at least equivalent in anti-inflammatory analgesic and antipyretic efficacy to comparator non selective NSAIDs in osteoarthritis, rheumatoid arthritis and other pain models (11). Emerging evidence suggests that rofecoxib may also find potential use as supportive therapy in various pathophysiologic conditions like Alzheimer's disease, and in various malignant tumors and polyps where Cox-2 is overly expressed (11). Merck and Co. Inc., mentioned that rofecoxib did not impair male fertility in rats at doses up to 100 mg/kg daily. So we found an important task to seek about the effectiveness and side effect profile of two doses of rofecoxib (Cox-2 inhibitor), in a long–term study (2 months), and on epididymal sperm characters and accessory sex glands in mature male rats.

MATERIALS AND METHODS

Animals: Twenty–four adult male albino rats, at 100 days of old and weights (200-400) g. They were housed in polypropylene cages under controlled conditions of temperature (24-26C°) and lighting (12 hours light/12 hours' dark), pelleted food and water were supplied ad libitum.

Experimental design: The study included two separate experiments. Each one consists of randomly divided two groups of animals (6 rats/group).

In experiment (1): Group 1 received distilled water served as control. Group 2 received rofecoxib at a dose of 0.35 mg/kg B.W. In experiment (2): Group 1 received distilled water served as control. Group 2 received rofecoxib at a dose of 0.71 mg/kg B.W. The doses of rofecoxib were calculated according to the doses used by humans (25, 50mg/70 kg/day), (12). All treatments were given daily for 60 days using gavages needle.

Sexual organs and accessory sex glands weights: Body weights at pre-and post treatments were recorded. At the end of experiments, animals were sacrificed by Ether administration. Abdominal cavity was then opened, Prostate, seminal vesicles, Right and left epididymis (head, body and tail) were dissected out and their weights were recorded.

Epididymal sperms count: Epididymal head sperms were counted according to Sakamoto and Hashimoto Procedure (13) using hemocytometer. Right epididymal head was dissected out, sectioned and its contents was squeezed in a clean water glass with the addition of 9.8ml of neutral buffer formalin and 0.1 ml eosin stain.

Percentage of live, dead and morphologically abnormal sperms: The percentage of live, dead and morphologically abnormal sperms were determined using eosin-nigrosin stained smears from the right epididymal tail dissected out, sectioned and its contents squeezed in a clean watch glass with the addition of 2ml of physiological normal saline at temperature (37C°), (14).

Histopathology: The testes were fixed in Bouins solution for 10 minutes then fixed in 10% neutral Buffered formalin for 24 hours. The fixed samples were dehydrated in alcohol and embedded in paraffin blocks. Serial sections of 4-5 micrometer were prepared, stained with hematoxylin and eosin (15) and examined under light microscope.

Statistical analysis: The data of each experiments were statistically analyzed using t-test. The level of significance was at $P< 0.05$ & $P<0.001$, respectively (16).

RESULTS

Oral administration of rofecoxib at a dose 0.35mg/kg, B.W., caused a significant (P<0.05) increase in weights of the head and body of epididymis, and increase in the percentage of dead /live sperms and sperm abnormalities with a significant (P<0.001) decrease in sperms count in the epididymal head and a significant increase (P<0.001) in the diameter of seminiferous tubules. Rofecoxib at the dose 0.71 mg/ kg B.W., caused a significant (P<0.05) increase in the percentage of dead/live sperms and sperm abnormalities and the diameter of seminiferous tubules (P<0.001).

Table (1): Effect of 60 days oral administration of rofecoxib (0. 71mg/kg B.W.) on weights of (body, testis, epididymis (head, body tail) and male accessory sex glands and epididymal sperm characters in rats.

Treatment		Control	Rofecoxib (0.71mg/kg B.W.)
Body weight (g)		292.3±17.6	332.6±19.8
Testis weight (mg/100g B.W)		476.3±11.3	442.3± 18.01
Epididymis weight	Head	76.16± 1.7	65.6± 6.9
	Body	15.4± 1.19	12.6± 2.4
	Tail	71.04± 2.4	63.09 ± 7.8
Prostate weight (mg/100g B.W)		198.14± 10.7	216.4± 4.44
Seminal vesicle weight (mg/100g B.W)		65.57±15.4	38.7±2.5
Percentage of dead/live sperms		1.74 ± 8.83	13.33±0.88 *
Percentage of morphologically Abnormal sperms		6.66 ± 0.66	22.33±2.858 ***
Epididymal headSperms×10^6 Sperm/ml)		$1.21 \times 10^6 \pm 0.09$	$1.06 \times 10^6 \pm 0.08$
Diameter of seminiferuos tubules (micron)		242.23± 4.234	266.73±5.586 ***

Numbers of animals 6 rats/group. Values are expressed as mean ± SE.Values with different letters are significantly different from control * P< 0. 05, *** P<0.001.

Table (2): Effect of 60 days oral administration of rofecoxib (0. 35mg/kgB.W) on weights of (body, testis, epididymis (head, body tail) and male accessory sex glands and epididymal sperm characters in rats.

Treatment		control	Rofecoxib (0.35mg/kg B.W.)
Body weight (g)		19.13 ± 210.16	195.2± 4.4
Testis weight (mg/100g B.W)		33.02 ±561.79	587± 17.8
Epididymis weight	Head	77.98 ± 6.103	99.08± 4.47 *
	Body	14.91± 1.407	22.6±2.83 *
	Tail	76.65± 7.84	77.02±8.74
Prostate weight (mg/100g B.W)		201.3± 14.12	189.16±22.07
Seminal vesicle weight (mg/100g B.W)		122.1 ± 77.18	39.4±2.87
Percentage of dead /live sperms		1.08± 3.83	15.4±1.32 ***
Percentage of morphologically Abnormal sperms		0.33 ±0.33	10.6 ± 0.87 ***
Epididymal headSperms×10^6 Sperm/ml)		1.14×10^6± 0.045	0.6×10^6 ±0.04 ***
Diameter of seminiferuos tubules (micron)		248.3± 5.55	279.96±6.243 ***

Numbers of animals 6 rats/group. Values are expressed as mean ± SE values with different letters are significantly different from control * P< 0. 05, *** P<0.001.

DISCUSSION

The significant increase in the epididymal head and body weights in animals treated with rofecoxib 0.35mg/kg B.W. could be explained on the basis of the inhibition of prostaglandin synthesis. Since the male reproductive tract is known to be a rich source of prostaglandins and the role of prostaglandins in the male reproduction has well documented (17). Intact and cultured rat epididymal

cells express cox-1 mRNA and Cox-2 mRNA (18), and the regulated secretion of electrolytes and fluids by the epithelial cell lining the epididymal tubules is an important process that leads to the formation of a correct milien for sperms maturation and storage (19). The secretion of anions and fluids is controlled by vasoactive peptides. The effects of these peptides can be blocked by NSAIDs (inhibitors of prostaglandin synthesis) (19). So, in the present study epididymal prostaglandin synthesis inhibition could lead to muscle relaxation, losing their ability to cause peristaltic contraction responsible for sperm transport to rate testis. Thus, tubules occluded by accumulation of dead, immobile sperms and increasing epidermal weight. This explanation was based on the observation of Hib and Oscar (20), that prostaglandin E and F enhance the contractility of smooth muscle layer surrounding epididymal tubule. Group treated with rofecoxib 0.71mg/ kg B.W., show a significant increase in the percentage of dead sperms and sperms abnormalities. In addition, rofecoxib at a dose 0.35mg/kg B.W., caused a significant increase in sperm abnormalities. These can be explained on the basis of prostaglandin inhibition which play important role in spermatogenesis (21) androgen secretion (22) sperm metabolism and function (23-25). PGs mediate normal pituitary hormonal secretion, interstitial cell stimulating (26), and follicle stimulating hormones (27), and prolactine (28), which all have a direct effects on spermatogenesis (29). Furthermore, Cox-2 expression is regulated by androgen (30). So, rofecoxib treatment could have a negative effect on androgen secretion which in turn may affect Sertoli cells and spermatogenesis (31). On the other hand, Kruase *et al*. (32), reported that specific Cox-2 inhibitor SC-236 has been shown to uncouple mitochondrial respiration and inhibit substrate oxidation and ATP turnover in rat mitochondria in vitro, such activity in sperm would probably decrease motility and viability, rofecoxib could have the same effect because it belongs to category selective cox-2 inhibitors. Prostaglandins and other arachidonate metabolites play an important role in the regulation of the hormonal secretions of the hypothalamic pituitary axis in vivo and in vitro (31). At the hypothalamic level PGE_2 synthesized by cyclooxygenase enzyme is an intracellular mediator of gonadotropin-releasing hormone (33,34). Indeed, it has been reported that cyclooxygenase (35), or lipo-oxygenas metabolities (36), or arachidonic acid (AA) itself (37), could be involved in the process of testosterone production from rat leydig cells which may account for the decrease in epididymal sperm count in animals treated with rofecoxib 0.35 mg/kg, B.W. On the other hand, it may be caused by testicular pathological changes caused by rofecoxib treatment as evidenced by degenerative changes in sertoli cells, sloughing of sertoli cells from the basement membrane losing its role in supporting nutrition and spermatogenesis (38). These changes may be responsible for the increase in the seminiferous tubules diameter caused by the two doses of rofecoxib.

In conclusion, rofecoxib treatment caused bad effect on epididymal sperm characters and fertility.

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