Effects of selected cyclo-oxygenase inhibitors on cardiovascular preparations in rabbits

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ABSTRACT

The present study aimed at shedding some light on the pharmacological effects of two NSAIDs (one selective, that is meloxicam, and the other is non-selective, that is piroxicam) on cardiac contractility and aortic smooth muscle of rabbit as a farm animal species. Rhythmic contractions of the heart were established using Gunn's apparatus (heart infusion assembly) and normal tone of the aortic rings was established using tension recording technique. The effects of graded increased concentrations of piroxicam and meloxicam on the installed organs were studied. The sites of action of piroxicam and meloxicam were also tried. The obtained results proved that piroxicam and meloxicam had concentration-dependant, negative inotropic and chronotropic effects on the rabbit's heart; and were without significant effects on aortic rings throughout the tested concentrations. The effects of piroxicam and meloxicam on the heart were not affected by pre-addition of atropine, and the effect of adrenaline was evident in presence of the tested drugs indicating the non-involvement of cholinergic and adrenergic pathways in their mechanism of action. The effects of the drugs might be then attributed to their direct effect on the cardiac myocytes probably via decreasing Ca^{2+} concentration. Data of the present study may indicate that piroxicam and meloxicam could be used effectively and safely in rabbits for their anti-inflammatory actions in small therapeutic doses. However, in large doses, they (particularly, piroxicam) may produce depressant effects on myocardial contractility that should be taken in consideration in case of introducing these drugs in therapy with larger doses.

Keywords: NSAIDs, piroxicam, meloxicam, anti-inflammatory, myocardial contractility, aorta, cardiovascular.

1. INTRODUCTION

Cyclo-oxygenase- (COX-) inhibitors are those drugs which have the ability to inhibit the activity of COX enzymes (types 1 and 2) resulting in inhibition of prostaglandin synthesis [1]. Two types of COX-inhibitors are established, the traditional non-selective non-steroidal anti-inflammatory drugs (NSAIDs) which block both types of COX. The second type is the selective COX-2 inhibitors which have no or minimal affinity, and thus, no effect on COX-1. The development of the COX-2 selective inhibitors was intended to provide drugs that would offer the same pain-relieving and anti-inflammatory effects as the traditional NSAIDs without causing the gastric ulcers and nephrotic effects that have been associated with the pioneer drugs [2].

NSAIDs can be categorized into many classes, including, salicylic acid derivatives (as aspirin), acetic acids (as indomethacin and diclofenac), propinoic acid derivatives (as naproxen and ibuprofen), oxicams (as piroxicam and meloxicam), pyrazolones (as phenylbutazone), and fenamic acids (as mefenamic acid). All groups of NSAIDs exert their effects by inhibiting one or both of the cyclo-oxygenase (COX) enzymes, COX-1 and COX-2 [3].

Application of NSAIDs has been utilized on a large scale in human therapeutics. However, they are still of less interest in veterinary practice in spite of its great importance. COX-inhibitors are very important in symptomatic treatment of disease conditions and for specific treatment as well. In symptomatic treatment,

they are prescribed in almost all cases together with the specific remedies to relieve variety of inflammatory symptoms such as fever, pain, swelling, congestion and edema. Moreover, they are the main or specific drugs used for treatment of chronic inflammatory conditions including rheumatism, rheumatoid, tendinitis, osteoarthritis, muscle aches, back aches, bursitis and menstrual cramps. In addition, they have special role post grafting to avoid graft rejection.

This study, therefore, was designed to demonstrate the pharmacodynamic profile of two NSAIDs (one selective, that is meloxicam, and the other is non-selective, that is piroxicam) in rabbit as a farm animal species, on the isolated cardiovascular preparations. This may shed some light on full characterization and utilization of this important group of drugs in veterinary medicine. The comparative profiles of piroxicam and meloxicam from the pharmacodynamic aspect of view has been also elucidated.

2. MATERIALS AND METHODS
2.1 Drugs
Piroxicam was obtained as the patent preparation Feldene® (Pfizer, USA) that is an intramuscular therapy for inflammatory conditions in man, formulated as 1 mL ampoules equivalent to 20 mg piroxicam. Meloxicam was obtained as the patent preparation Mobic® (Boehringer Ingelheim, Germany) that is an intramuscular therapy for inflammatory conditions with minimal gastric side effects, formulated as 1.5 mL ampoules equivalent to 15 mg meloxicam.

For the in vitro studies, two-fold serial dilutions (from 0.5 to 200 µg/mL cannula or bath of piroxicam and 0.5 to 100 µg/mL cannula or bath of meloxicam were made from the stock drugs using an appropriate physiological salt solution (see below).

2.2 Experimental animals
Male New Zealand white rabbits were used for studying the comparative effects of piroxicam and meloxicam on contractility patterns of cardiovascular preparations. Adult animals were purchased from a local farm in Tripoli, Libya and were of body weight ranging between 1.8 and 2 Kg. Animal care was in accordance with Guidelines for Care and Use of Laboratory Animals in Biomedical Research of the National Institutes of Health of the United States [4], and approved by our institutional committee of Experimentation Ethics on Animal Use.

2.3 Chemicals
Adrenaline was purchased from Aguettant (Lyon®, France) and Atropine sulphate was purchased from Sigma-Aldrich® (Saint Louis, USA). All other classical chemicals were purchased from local distributors and were of analytical grade.

2.4 Gunn's apparatus
The apparatus consisted of a bottle clamped to an upright and connected downward by a rubber tube to a central glass spiral embedded in a water bath electrically heated. To the other end of the glass spiral, a glass cannula (20 ml. capacity) was attached through a short tube. Two light pulleys were used to direct the thread to the transducer connected to Kymograph (Griffin and George Ltd.) its speed was 2.5 mm/second. Oxygen (from oxygen cylinder) was supplied to perfuse Ringer-Locke's solution through a rubber pipe. Effects of graded increased concentrations of piroxicam and meloxicam were demonstrated. The sites of action of piroxicam and meloxicam are also investigated.

2.5 Glass jar bath
An organ bath (model GRAZ, type 846, Hugo Sachs Elektronik, HSE, D-79232 March, Germany) was used. The Graz organ bath consisted of a plexiglass base plate with 2 vertical columns, each of which carries organ vessel, tissue carrier and lever transducer (B40, type 373, serial number 07416, HSE), amplifier (TAM-A type 705/1, HSE) and digital recording unit (Data translator, DT 9800 BNC translator, Box 16SE serial no. 20072401, HSE) with data acquisition software for measuring the contraction forces. Organ vessels were of 10 ml capacity. The organ vessels carry a drain cock for draining the solution. Fresh solution which must be pre-warmed and aerated was introduced into the vessel with a syringe of appropriate size. A frit was fused into the base of each organ vessel for aerating the solution. A needle valve was provided for separately adjusting the aeration rate of each vessel. The standard tissue carrier was suitable for mounting aortic rings.

2.6 Physiological salt solutions
Ringer-Locke's physiological salt solution for the heart and Krebs' solution for the aorta were prepared as indicated by [5] with the following compositions: Sodium chloride (9.00 g), Potassium chloride (0.42 g), Calcium chloride (0.24 g), Sodium bicarbonate (0.15 g), Glucose (1.00 g) and Distilled water to (1000.00 ml) for Ringer-Locke's; and Sodium chloride (8.00 g), Potassium chloride (0.20 g), Calcium chloride (0.20 g), Magnesium chloride (0.10 g), Sodiumdihydrogen phosphate (0.05 g), Sodium bicarbonate (1.00 g), Glucose (1.00 g) and Distilled water to (1000.00 ml) for Krebs' solution.

2.7 Heart infusion assembly
A rabbit under investigation was humanely slaughtered. The chest was opened and the pericardium was carefully picked up and opened for exposing the heart. The heart was then removed with a part of aorta and placed in a Petri dish containing warm oxygenated Ringer-Locke's solution. It was gently squeezed with fingers to remove the blood from it. The cannula of the apparatus was introduced in and tied to the aorta; by such way the cardiac preparation was fixed to the apparatus. A transducer was attached to a thread passed through the two light pulleys and tied to a platinum hock that was fixed to the wall of the left ventricle at its apex. Perfusion of the heart with warm oxygenated Ringer-Locke's solution at 37 °C was carried out until the rhythmic contraction of the heart

was obtained. Then the method explained by [6] using Gunn's apparatus was used for studying the effects of graded increased concentrations of piroxicam and meloxicam on rabbit's heart. The sites of action of piroxicam and meloxicam were also investigated.

2.8 Tension recording technique

The method explained by [7] was used for studying the effect of piroxicam and meloxicam on rabbit's aortic ring preparations. The aorta was obtained from a freshly slaughtered rabbit and gently cleared from blood by gentle perfusing with the Krebs' solution. Then the aorta was cut into 0.5 cm-long rings with care. A ring preparation was suspended in the organ bath containing Krebs' solution at 37 °C where two threads were passed through the ring, one was fixed to the fixation tissue carrier and the other was connected to an isotonic transducer, amplifier and digital recording unit. The rings were subjected to a resting tension of 1.0 g and were allowed to equilibrate for approximately 60 minutes by the change of bath solution every 15 minutes. The effects of graded increased concentrations of piroxicam and meloxicam were demonstrated with minimal rest period of fifty minutes was allowed for full recovery between the successive additions.

2.9 Statistical analysis

Data were expressed as the mean ± SEM of *n* observations, where *n* represents the number of tissues studied in each experiment (triplicates). The data were analysed using one-way analysis of variance (ANOVA) followed by LSD post-hoc test for multiple comparisons among the untreated tissue segments and those treated with different drug concentrations (0.5~200 µg/ml bath). Differences were considered significant at P ≤ 0.05. Percentage of drug-induced inhibitions was also calculated. All statistical procedures were calculated using GraphPad Prism software version 6 for Windows (GraphPad Software, La Jolla, California, US).

3. RESULTS AND DISCUSSION

In veterinary use, there is support to the use of NSAIDs for the control of pain-associated veterinary procedures such as dehorning and castration. However, as different species have varying reactions to different medications in the NSAID family, little of the reported research data can be extrapolated to animal species other than those specifically studied. For example, ketoprofen effects have been studied in horses more than in ruminants but, due to controversy over its use in race horses, veterinarians who treat livestock in the United States more commonly prescribe flunixin meglumine, which, while labelled for use in such animals, is not indicated for post-operative pain. In the United States, meloxicam is approved for use only in canines, whereas (due to concerns about liver damage) it carries warnings against its use in cat [8]. In spite of these warnings, meloxicam is frequently prescribed "off-label" for non-canine animals.

Among most important NSAIDs that have been used expensively in human filed and not yet applied in parallel manner in veterinary field are piroxicam (long acting nonselective COX blocker), and meloxicam (intermediate acting selective COX2 blocker). It is important from the pharmacological point view to describe different actions including either pharmacological or side ones in different animal species.

The present work was performed to investigate some pharmacodynamic effects of piroxicam and meloxicam in rabbits as a farm animal model in vitro, namely, the effects of piroxicam and meloxicam on isolated cardiovascular preparations.

The effects of graded increased concentrations of piroxicam and meloxicam on the contractility of rabbit's heart were recorded in table (1). Piroxicam at concentrations up to 4 µg/ml cannula had no effect on the cardiac contractility. A minimal inhibitory effect was observed at concentration of piroxicam of 8 µg/ml cannula that produced about 12% inhibition of cardiac contractility. Complete relaxation of the cardiac muscle was produced after addition of piroxicam at concentration of 128 µg/ml cannula. Meloxicam at concentrations up to 8 µg/ml cannula had no effect on the cardiac contractility. The minimal effect was recorded at concentration of 16 µg/ml cannula that produced about 22% inhibition of cardiac contractility. Complete relaxation of the cardiac muscle was established after addition of meloxicam at concentration of 100 µg/ml cannula.

Table 1: Effects of piroxicam and meloxicam on isolated rabbit's heart.

Concentrations (µg/ml bath)	Response of rabbit ̓s heart.			
	Piroxicam (Amp., g)	Piroxicam (inhibition %)	Meloxicam (Amp., g)	Meloxicam (inhibition %)
Pretreated	1.271±0.088	0.000±0.000	1.000±0.000	0.000±0.000
0.5~4	1.271±0.088	0.000±0.000	1.000±0.000	0.000±0.000
8	1.033±0.088	11.500±2.676*	1.000±0.000	0.000±0.000
16	0.800±0.115	29.600±7.189*	0.867±0.033*	21.667±1.667*
32	0.533±0.145*	55.500±9.179*	0.467±0.033*	54.000±2.309*
64	0.067±0.033*	94.667±2.667*	0.300±0.000*	72.667±1.453*
100	---	---	0.000±0.000*	100.000±.000*
128	0.000±0.000*	100.000±0.000*	---	---

*P ≤ 0.05, significantly different from pre-treated

Trials were performed to locate the site of action of piroxicam and meloxicam on the rabbit's heart. Concentrations of the drugs that produced submaximal or maximal inhibitory effects were used in such experiments. To investigate the hypothesis that piroxicam and meloxicam produce their inhibitory effects on the cardiac muscle preparations via $\beta 1$-receptor, adrenaline (Adr., adrenergic stimulant) was used, where it produced its stimulatory effects in spite of pre-addition of either piroxicam or meloxicam (figure 1A & 2A). As for cholinergic involvement, both drugs produced, individually, their inhibitory effects on cardiac muscle despite pre-exposure to atropine (atrop., muscarinic receptor blocker), (figure 1B & 2B).
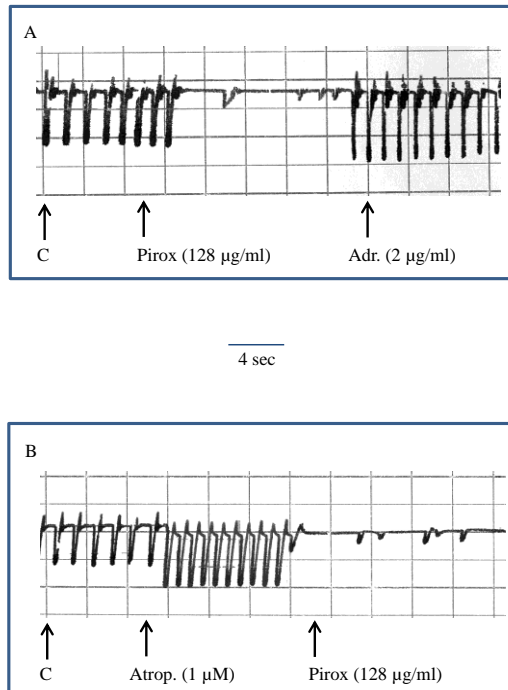

Figure 1: (A) 128 µg/ml cannula piroxicam (pirox.) followed by 2 µg/ml cannula adrenaline (Adr.).
(B) 1 µM atropine sulphate (Atr.) followed by 128 µg/ml cannula piroxicam (pirox.).
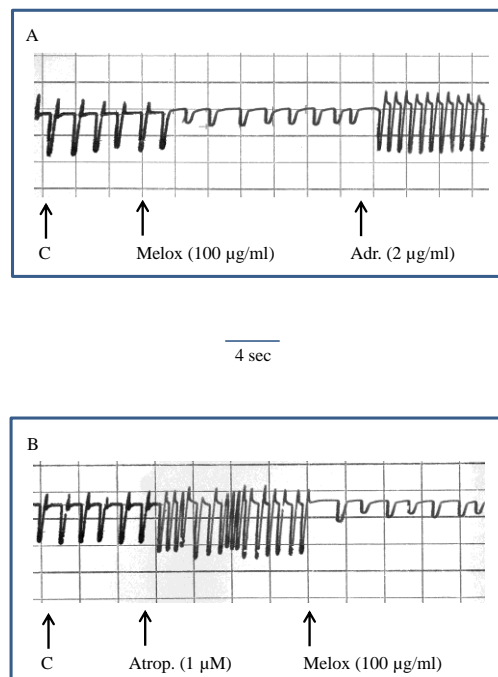

Figure 2: (A) 100 µg/ml cannula meloxicam (melox.) followed by 2 µg/ml canula adrenaline (Adr.).
(B) 1 µM atropine sulphate (Atr.) followed by 100 µg/ml cannula meloxicam (pirox.).

The effects of piroxicam and meloxicam on vascular smooth muscles on rabbit's aortic rings were shown in figure (3); it has been found that addition of graded increased concentrations of piroxicam and meloxicam up to 200 and 100 µg/ml bath, respectively, had no significant effects on the smooth muscle of the aorta.
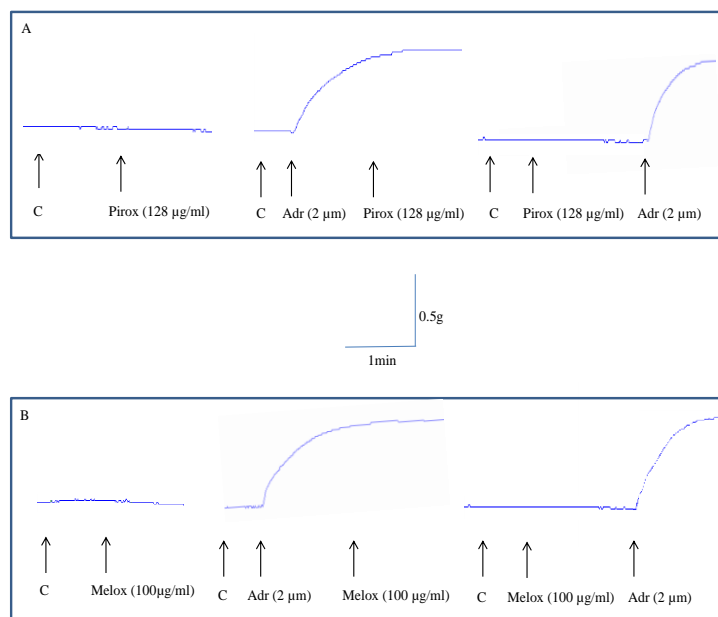

Figure 3: (A) Effect of piroxicam (pirox.) before and after adrenaline
(B) Effect of meloxicam (melox.) before and after adrenaline

The obtained results of this study on the cardiovascular muscles proved that, piroxicam and meloxicam had a negative inotropic and chronotropic effects on the rabbit's heart. Piroxicam and meloxicam produced a direct and concentration-dependant depression of the myocardial contractility. These negative inotropic and chronotropic effects of the tested drugs were not referred to either ß1 adrenergic blocking or cholinergic stimulant effects, as adrenaline (2 µg/ml cannula) was able to produce its cardiac stimulatory effect in presence of piroxicam (128 µg/ml cannula) and meloxicam (100 µg/ml cannula). Also, the negative effects of both drugs were still evident in presence of atropine (1 µM).

Contraction of the cardiac myocytes is believed to be dependant upon the intracellular concentration of available calcium ions in the vicinity of the contractile apparatus [9]**.** So, the direct myocardial depressant effect of piroxicam and meloxicam in the present work might be attributed somehow to a modification of calcium function.

It was observed that, the two tested drugs had no effects on the resting smooth muscle of aortic ring preparations. Also, in the presence of piroxicam and meloxicam, adrenaline was able to produce its stimulatory effect, thus both drugs appeared to have no α1-adrenergic blocking effects on the isolated rabbit's aorta. In a third trial, piroxicam and meloxicam were not able to relax the adrenaline-pre-contracted aortic ring preparations.

The present data may be in accordance with [10]**,** who reported that NSAIDs are frequently prescribed in elderly patients for several rheumatological and nonrheumatological indications. Numerous adverse reactions including a causal relation between the use of NSAIDs and the onset of congestive heart failure (CHF). The pathophysiology of CHF and the pharmacological properties of NSAIDs support this hypothesis. In particular, the inhibition of prostaglandin synthesis may adversely affect cardiovascular homeostasis in patients with a propensity to develop CHF.

The findings of our study may be not in accordance with [11]**,** who found that celecoxib induced relaxation of rat's aortic rings and that relaxation was attenuated by the nitric oxide synthase inhibitor NG-nitro-l-arginine methyl ester (l-NAME, 10^{-4} M) and by the guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ, 10^{-5} M). In aortic rings, celecoxib (3×10^{-5} M) caused a fivefold increase in the cGMP level and potentiated that induced by sodium nitroprusside (5×10^{-7} M). Celecoxib and valdecoxib inhibited human PDE5A1 with an IC50 of 1.6×10^{-5} and 1×10^{-4} M, respectively, whereas other coxibs were without inhibitory effect. The authors implied that these unexpected findings clearly support the notion that celecoxib possesses an as yet undisclosed molecule-specific property that possibly compensates a decrease of prostacyclin-dependent cAMP generation by concomitantly increasing cGMP levels resulting from inhibition of PDE5. The difference between this result and that recorded in the present study may be attributed to the difficulty of getting an endothelium-

intact preparations due to the damage of endothelium in rings during flushing, cutting and fixation.

Data of the present study may be also inconsistent with that recorded by [12], who studied the effects of diclofenac, piroxicam and celecoxib on the rat aortic rings with intact endothelium and spiral strips of rabbit's portal vein. The isolated tissues were stimulated with 1 μM of norepinephrine. Diclofenac 15 and 20 μM and piroxicam 15 μM produced significant relaxation in rat aortic ring while there was no significant effect of celecoxib. Diclofenac 10, 15 and 20 μM, piroxicam 5 μM, celecoxib 5, 10 and 20 μM produced significant vasorelaxant effects on rabbit's portal vein. The authors concluded that only nonspecific COX inhibitors affect arteriolar tone while venous tone is also decreased significantly not only by nonspecific but also by specific COX-2 inhibitors. Discrepancy between these results and ours may be attributed to absence of intact endothelium in our ring preparations.

4. CONCLUSION

From data of the present study, it could be concluded that piroxicam and meloxicam may produce depressant effects on the cardiac contractility; and were without effects on aorta. These cardiac side actions should be taken in consideration in case of introducing these drugs in therapy. Both piroxicam and meloxicam could be used in rabbits with small therapeutic doses for short courses to get the benefits of their anti-inflammatory actions and avoid their adverse actions.

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