EXPERIMENTAL STUDY

The effect of meloxicam and deendothelisation on vascular responses in the rabbit renal and ear arteries

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Abstract

Background: **The use of non-steroidal anti-inflammatory drugs (NSAIDs) is frequently limited by adverse effects resulting from the disruption of homeostatic functions of prostaglandins.**
Objectives: **This study was aimed at the evaluation of the effect of selective COX-2 inhibitor meloxicam on vasoconstrictor responses to noradrenaline (NA) in rabbit renal artery chosen as a model vessel and in rabbit ear artery as a peripheral artery under in vitro conditions.**
Methods: **Rabbit renal and ear arteries were perfused at constant flow. Vascular responses to NA before and after meloxicam administration and after deendothelisation by air bubbles were measured and registered as changes in perfusion pressure.**
Results: **It was found out that vasoconstrictor responses to noradrenaline when exposed to meloxicam were not enhanced significantly in both arterial preparations. Deendothelisation itself did not increase responses affected by meloxicam in the renal and ear arteries but in comparison with control groups the responses were significantly augmented, especially in the ear artery.**
Conclusions: **The results obtained in this study demonstrate that meloxicam had not affected adversely the vasoconstrictor activity in different types of vessels without selectivity on vascular beds. Endothelial removal potentiated vasoconstrictor responses in arteries pre-treated by meloxicam when compared with intact vessels.** ***(Tab. 2, Fig. 4, Ref. 19.)***
Key words: renal and ear arteries, non-steroidal anti-inflammatory drugs, vasoconstrictor responses, deendothelisation.

Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat acute and chronic inflammatory disease and pain. This pharmacological group belongs to the most frequently used drug therapy in the adult population. The use of NSAIDs is frequently limited by adverse effects resulting from the disruption of the homeostatic functions of prostaglandins (PGs). PGs act as modulators of physiologic functions in the kidney, and therapy with NSAIDs may affect the renal physiology (Brater, 1999). The most common adverse effects of NSAIDs are gastrointestinal erosions and bleeding. However, renal effects, including salt retention and (rarely) acute renal failure, can also occur (Harris, 2002).

NSAIDs act by inhibiting cyclooxygenase (COX), the rate-limiting enzyme in metabolic conversion of arachidonic acid into prostanoids. There are 2 isoforms of the COX enzyme that have been identified, and both are inhibited by conventional NSAIDs. COX-1 is expressed constitutively in most tissues and is thought to play an important function in the protection of gastric mucosa and hemostasis. COX-2 was identified as an enzyme induced in inflammatory states and tumorigenesis. However, it is also expressed constitutively in brain and renal tissues. Despite some recognized overlapping between the expression patterns and putative functions of the 2 isozymes, the relatively ubiquitous expression of COX-1 and induction of COX-2 in pathophysiologic states led to the formulation of the COX-2 hypothesis, stating that specific inhibition of COX-2 will preserve therapeutic benefits of NSAIDs but will not interfere with homeostatic functions of prostanoids (FitzGerald and Patrono, 2001).

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Acknowledgements: This study was supported by grants VEGA 1/6136/99 and 1/9302/02, Grant Agency for Science, Ministry of Education of the Slovak Republic.

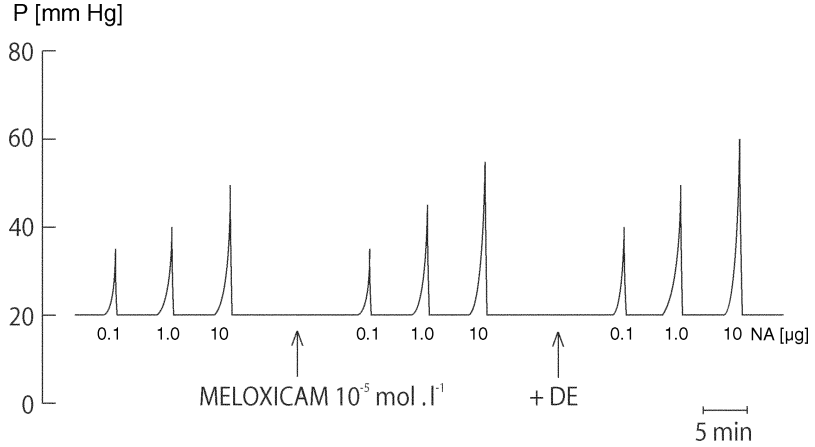

Fig. 1. Responses of an isolated renal artery to noradrenaline before and after meloxicam administration and after deendothelisation. Illustration of experiment. NA — noradrenaline, DE — deendothelisation, P — perfusion pressure.

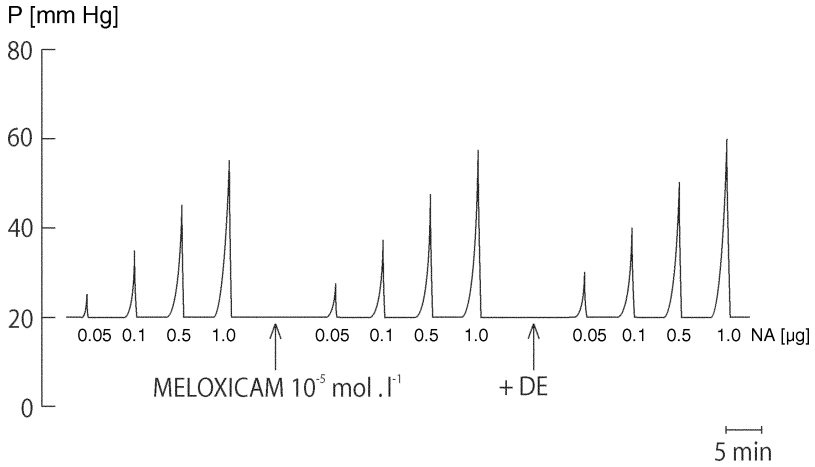

Fig. 2. Responses of an isolated ear artery to noradrenaline before and after meloxicam administration and after deendothelisation. Illustration of experiment. NA — noradrenaline, DE — deendothelisation, P — perfusion pressure.

In our previous experiments we evaluated vasoconstrictor responses to selected NSAIDs (indomethacin, ibuprofen, phenacetin) in rabbit renal and femoral arteries (Kristová et al, 2002). These drugs inhibit both COX isoforms resulting in an increase in vasoconstrictor activity. Therefore, the aim of the present work was to study the effect of selective COX-2 inhibitor meloxicam on vasoconstrictor responses to noradrenaline in rabbit renal artery chosen as a model vessel and in rabbit ear artery as peripheral artery under in vitro conditions.

Materials and methods

Rabbits (Cincilla) of both sexes weighing 2.5–3.0 kg were used in these experiments. The animals were sacrificed by cervical dislocation. The following experimental procedures were used:

Isolated renal artery: Renal arteries were excised together with the kidneys and placed in Tyrode's solution. After isolation, polyethylene cannules were introduced into each artery, fixed with cotton thread, and the vessel segment was cut up to achieve the constant length of 5 mm. Two paired vessel segments from the same animal were placed into the perfusion system and perfused parallel at a constant flow with Tyrode's solution (the composition in mM: NaCl 137.0, KCl 2.7, $MgCl_2$ 1.1, NaH_2PO_4 0.32, $CaCl_2$ 0.9, $NaHCO_3$ 11.9, glucose 5.5). The perfusion solution was saturated with 95 % O_2/5 % CO_2, maintained at 37 °C and at pH 7.25–7.35. Changes in pressure in the perfusion system were detected by means of tensometric transducer (LDP 102) and registered on a recorder (TZ 4200, Tesla). After the equilibration period of 30 minutes, the basal perfusion pressure was adjusted at 14–18 mmHg with a flow rate of 22 ml/min.

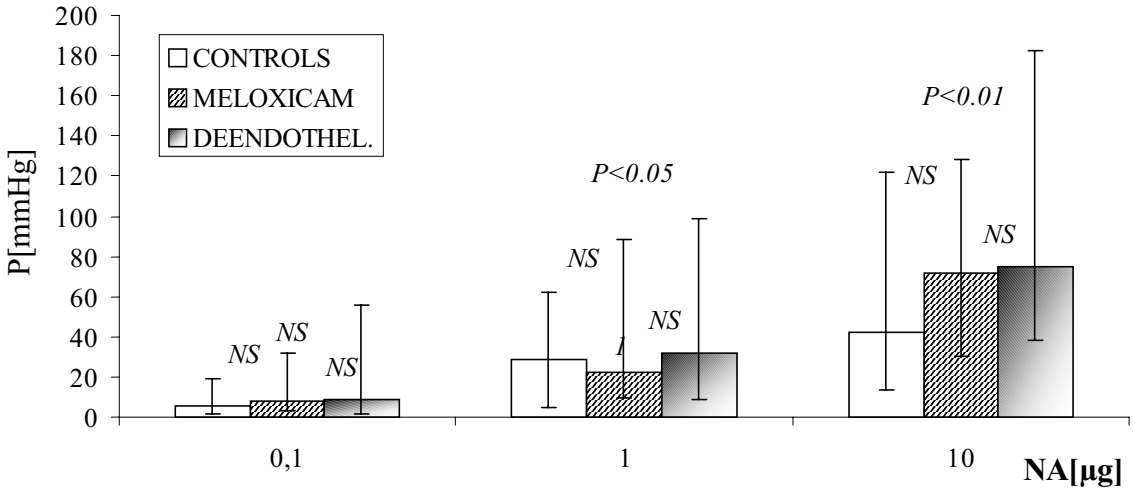

Fig. 3. The effect of meloxicam and deendothelisation on vasoconstrictor responses to noradrenaline in the rabbit renal artery (n=7). All values expressed as medians and their confidence limits. NA — noradrenaline.

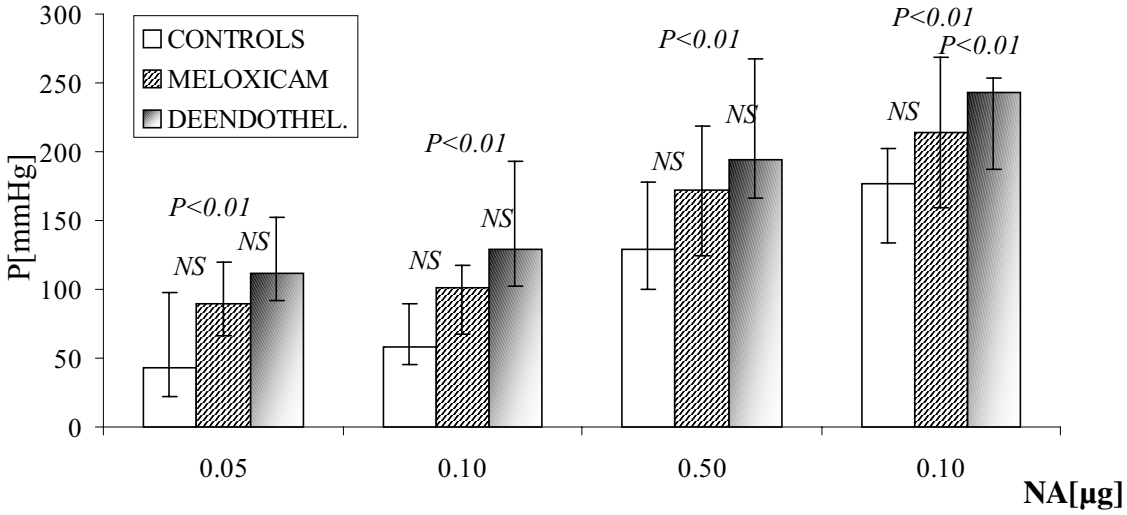

Fig. 4. The effect of meloxicam and deendothelisation on vasoconstrictor responses to noradrenaline in the rabbit ear artery (n=10). All values expressed as medians and their confidence limits. NA — noradrenaline.

Isolated ear artery: The ears were cut off and both central ear arteries were carefully removed and placed in Tyrode's solution. After the isolation and cannulation the vessel segments were transferred into a vessel chamber and perfused at similar conditions as described above (the basal perfusion pressure: 20–25 mmHg, flow rate 3 ml/min).

Experimental protocol: Both the renal and ear arteries were handled identically. After the equilibration, the standard doses of noradrenaline 0.1; 1.0; 10 µg in the case of renal artery and 0.05; 0.1; 0.5; 1.0 µg in the ear artery were injected directly into the cannula in a volume of 0.1 ml at intervals of approximately 5 minutes. Then the vessel segments were continuously perfused during a period of 15 minutes with meloxicam in concentration of 10^{-5} mol.l^{-1} followed by the same doses of noradrenaline. After the latter period, deendothelisation by air bubbles lasting 2 min in the renal artery and 10 min in the ear artery was performed, and the same doses of noradrenaline were used to evaluate the vasoconstrictor responses of vessel segments. The experimental protocol is illustrated in Figs 1 and 2.

Drugs: Noradrenaline hydrogentartarate (Léčiva), meloxicam (Boehringer Ingelheim). Meloxicam was dissolved in 1 ml of ethanol. For dilution of drugs, Tyrode's solution was used. All solutions were prepared fresh before each experiment.

Statistical evaluation of results: The data (expressed in mmHg) were evaluated using Wilcoxon–Wilcox test for multiple comparisons of dependent samples.

Tab. 1. The effect of meloxicam and deendothelisation on vasoconstrictor responses to noradrenaline in the rabbit renal artery (n=7). All values expressed as medians and their confidence limits in mmHg. NA — noradrenaline, DE — deendothelisation, NS — non-significant.

Dose of NA	Control	Meloxicam	DE	p_{contr} vs mel.	$p_{mel.}$ vs de	$p_{contr.}$ vs de
0.1 µg	5.25 (1.50;18.75)	8.25 (3.00;32.25)	9.00 (1.50;55.50)	NS	NS	NS
1.0 µg	28.50 (4.50;62.25)	22.50 (9.75;88.50)	31.50 (9.00;99.00)	NS	NS	<0.01
10 µg	42.0 (13.50;112.25)	72.0 (30.00;128.25)	75.0 (38.25;182.25)	NS	NS	<0.01

Tab. 2. The effect of meloxicam and deendothelisation on vasoconstrictor responses to noradrenaline in the rabbit ear artery (n=10). All values expressed as medians and their confidence limits in mmHg. NA — noradrenaline, DE — deendothelisation, NS — non-significant.

Dose of NA	Control	Meloxicam	DE	p_{contr} vs mel.	$p_{mel.}$ vs de	$p_{contr.}$ vs de
0.05 µg	42.75 (22.05;97.50)	89.63 (66.00;120.00)	111.75 (92.25;152.25)	NS	NS	<0.01
0.10 µg	57.75 (45.75;90.00)	101.25 (67.50;117.75)	129.38 (102.00;193.50)	NS	NS	<0.01
0.50 µg	128.63 (99.75;177.75)	172.50 (124.50;218.25)	194.25 (166.50;267.00)	NS	NS	<0.01
1.00 µg	176.63 (134.25;202.50)	214.50 (159.75;268.50)	243.38 (186.75;253.50)	NS	<0.01	<0.01

Results

Both renal and ear arteries were contracted with noradrenaline in a dose-dependent manner under in vitro conditions. When exposed to meloxicam (10^{-5} mol.l^{-1}) noradrenaline-induced contractions were not enhanced significantly in both arterial preparations (Figs 3 and 4).

Deendothelisation by air bubbles did not significantly affect the vasoconstrictor responses of both arterial preparations to noradrenaline when compared with meloxicam effect. The vasoconstrictor responses of renal and ear arteries to noradrenaline in the presence of meloxicam and after deendothelisation are shown in Figs 3 and 4. All numerical values expressed as medians and their 95 % confidence limits (CL) with statistical evaluation of multiple comparisons between control, meloxicam and deendothelisation groups in renal and ear arteries are shown in Tables 1 and 2.

The most pronounced effect of deendothelisation was observed in the ear artery where significant increase in all responses to noradrenaline was found when compared with controls (Fig. 4) whereas in the renal artery there were potentiated responses to higher noradrenaline doses of 1.0 and 10 µg (Fig. 3).

Discussion

The results of this study showed that vasoconstrictor responses to noradrenaline were not enhanced significantly by meloxicam in both rabbit renal and ear arteries. Deendothelisation itself did not increase significantly the responses affected by meloxicam in renal and ear arteries but in comparison with control groups the responses were significantly augmented, especially in the ear artery.

In vitro studies with human tissues have confirmed the high affinity of meloxicam for COX-2, whereas COX-1 was inhibited only at highest concentrations. The pharmacodynamic data indicate that the selectivity of meloxicam for COX-2 is inferior to that displayed by rofecoxib, but superior to that demonstrated by other conventional NSAIDs such as indomethacin, ibuprofen or naproxen (Tacca et al, 2002).

NSAIDs administration has been associated with the occurrence of renal adverse events including fluid and electrolyte disturbances, tubulointerstitial nephritis, papillary necrosis, glomerular lesions and acute renal failure (Perazella and Eras, 2000). In most cases, these complications are caused by NSAID-induced reduction in prostaglandin synthesis. It is known that in pathological conditions such as congestive heart failure, hepatic cirrhosis, hypovolaemia, nephrosis or chronic renal impairment, the maintenance of renal blood flow and glomerular filtration are dependent on the compensatory activity of local COX-derived prostaglandins. COX inhibition induced by NSAIDs can impair this compensatory mechanism and facilitate the occurrence of acute renal failure (Schlondorff, 1993). Studies in animal models and clinical investigations have demonstrated that COX-1 and COX-2 are constitutively expressed in the kidney, and both isoforms are likely to participate in renal function regulation (Yang et al, 1998). However, the role of selective versus

preferential inhibition of COX-2 in inducing renal disorders is still unclear. According to the recent analysis of data from the US FDA Adverse Event Reporting System and from published case reports, selective COX inhibitors have the same potential for renal toxicity as non-selective NSAIDs (Ahmad et al, 2002).

In addition to vasodilator prostaglandins (PGE_2, PGI_2), there is an increasing evidence that endothelial products such as nitric oxide (NO) are involved in the control of renal function (Gonzáles et al, 1998). The results obtained in this study suggest that the renal hemodynamic and excretory functions may be more sensitive to the prolonged cyclooxygenase inhibition, when NO production is reduced. The inhibition of NO synthesis has been found to potentiate the effect of several vasoconstrictors, such as 5-hydroxytryptamine, endothelin-1, thromboxane A^2 - analogue U46619 (Gude et al, 1998), noradrenaline (Kristová et al, 1999) in different vascular beds, leading to general vasoconstriction and blood pressure increase (Rees et al, 1989, Gardiner et al, 1990). Therefore, the interaction of both arachidonate and NO pathways may play an important physiological role in the regulation of vascular tone (Di Rosa et al, 1996).

The results of our experiments did not reveal any unfavorable effects of meloxicam on vasoconstrictor activity in both types of vessels and showed no selectivity in different vascular beds. The removal of the endothelium as the source of vasodilating substances led to the potentiation of vasoconstrictor responses when compared with controls, the fact of which is in agreement with our previous findings (Kristová et al, 1993). If endothelium was damaged or functionally affected by the inhibition of NO synthase, such conventional NSAIDs as indomethacin could potentiate the responses to vasoconstrictors (Kristová et al, 2000). Increased responses to vasoconstrictor stimuli after deendothelisation were reported by Carrier and White (1985) and Tesfamariam et al (1992). It is likely that the decreased production of vasodilator factors by endothelium such as NO and PGI_2 may enhance the readiness of vascular wall to the effect of vasoconstrictors.

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Received November 28, 2003.
Accepted December 19, 2003.