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Oral misoprostol does not protect the kidneys from diclofenac induced toxicity: data from an unilateral ureteral obstructive rat model

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Abstract. – OBJECTIVE: Ureteral obstruction leads to permanent changes in the structure of the kidney by several mechanisms. In this study, it was hypothesized that there would be a protective effect of misoprostol against diclofenac in rats with unilateral ureteral obstruction (UUO).

MATERIALS AND METHODS: Twenty-two female rats were randomized into 5 groups of 4 and 2 rats for the control group. The right ureter was sutured. The rats were grouped as control, contrast agent, contrast agent +N-acetylcysteine (NAC), diclofenac and diclofenac + misoprostol groups.Radiographic contrast agent was given iv on the 3rd day and other agents were administered orally for 1 week. The rats were sacrified after 1 week and histopathological and biochemical oxidative stress markers were evaluated.

RESULTS: The contrast agent and NAC group had lower rates of hemorrhage, inflammation, obstructive dilatation and fatty degeneration compared to the contrast agent only group ($p < 0.05$). No differences were seen in the normal kidneys. Between all the groups, there was no difference for tubule epithelium damage ($p > 0.05$). The contrast agent and NAC group had higher rates of antioxidant SH level compared to the contrast agent only group ($p < 0.05$) and lower rates of oxidative end product carbonyl groups ($p < 0.05$). For normal kidneys no difference was seen. No statistical difference was seen in MDA levels ($p > 0.05$). Statistically no difference was seen between the diclofenac group and the diclofenac and misoprostol group neither pathologically nor chemically ($p > 0.05$).

CONCLUSIONS: These results showed that NAC is protective against radiographic contrast agent toxicity when given before and after administration in obstructed kidneys as in previous data. Misoprostol was not observed to have any protective effect against diclofenac in obstructed kidneys.

Key Words:
Misoprostol, Diclofenac, Kidney, Toxicity.

Introduction

Ureteral obstruction produces tubular atrophy and cell death in the kidney. Apoptosis is reponsible for tubular cell death. This is normally involved in postnatal development and tissue renewal in adults. When rat kidneys are obstructed, renal tubular cell apoptosis begins in about 4 days and peaks after 15 days, with interstitial cell apoptosis continuing for the duration of the obstruction[1]. Another toxic effect comes from the accumulation of oxidative stress products. Ureteral obstruction also leads to progressive and eventually, permanent changes in the structure of the kidney, including the development of tubulointerstitial fibrosis and interstitial inflammation2.

In adults, the most common cause of unilateral ureteral obstruction (UUO) is renal lithiasis, which causes a sudden blockage of one ureter and leads to an acute obstruction[3]. Many renal colic patients with various levels of ureteral obstruction are seen in daily clinical practice and excretory urography is applied as it has been considered the "gold standard" for the evaluation of the upper urinary tract for many years. Radi-

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ographic contrast agents are used and non-steoroidal anti-inflammatory drugs (NSAIDs), mostly diclofenac for pain relief, are prescribed.

Misoprostol, a prostaglandin E1 analogue, is widely used in preventing NSAID-induced gastric ulcers and prolongs the survival of cardiac and kidney transplantation, synergizes cyclosporine, and protects against cyclosporine-induced renal damage. Based on the above reasons, we hypothesized that misoprostol could be used against diclofenac induced renal toxicity in UUO[4].

This work was designed to investigate the effects of misoprostol effects on diclofenac induced toxicity in UUO rat model while using N-Acetylcysteine (NAC) for study design approval which has known protective effects from radiographic contrast agents. A period of 1 week of obstruction was selected because when there are enough pathological changes for comparison, biochemical changes can also be discriminated. Oxidative stress is the main pathway of UUO renal damage. Some markers which have been studied have shown oxidative stress, such as lipid peroxidation marker malondialdehyde (MDA), protein degradation marker carbonyl assay. High antioxidant thiol (SH) levels are protective showing less oxidative stress[5].

Materials and Methods

Study Design

The study comprised 22 healthy Wistar albino female rats weighing 250-300 g. The rats were randomized into 5 groups of 4 and 2 for the control group. The rats were kept at normal room temperature (22 °C) and 50% humidity preoperatively and in cages postoperatively. General anesthesia with ketamine HCl (40 mg/kg, Parke Davis, Detroit, MI, USA) and Xylazine HCl (10 mg/kg, Bayer, Wuppertal, Germany) was administered. The incision site was shaved and sterilized with povidine-iodine.

A midline incision was made to reach the right kidney and right ureter at the level of the lower pole. The right ureter was sutured at the lower pole level with 4\0 vicryl (Coated Vicryl Plus®, Ethicon, Somerville, NJ, USA) while other structures were protected. The laparatomy incision was closed in the anatomical base.

The rats were separated into 5 groups: Group 1, control, Group 2, contrast agent, Group 3, contrast agent + NAC, Group 4, diclofenac and Group 5, diclofenac + misoprostol (Table I).

Radiographic contrast agent was given on the 3rd day intravenously, and other agents were administered orally for 7 days (Table II). The rats were sacrified on the 7th day after the UUO. Histopathological and biochemical oxidative stress markers (lipid peroxidation, oxidative protein damage, antioxidant level) were studied in the obstructed and the normal kidneys of the rats.

Histopathological Examination

Pathological examination was performed by a pathologist blind to the samples using a light microscope. Tissue samples from both the obstructed and normal kidneys of the rats were taken and were embedded in paraffin blocks. 5 μm sections were taken, deparaffinized and dyed with hematoxylin-eosin. The samples were evaluated at x40 magnification. Hemorrhage, inflammation, obstructive dilatation, fatty degeneration and tubule epithelium damage were evaluated.

Biochemical Evaluation

Tissue samples were kept at –70 ºC until the analysis day. The kidney samples were weighed and homogenized with 1\10 rate 0.15M KCL.

Malondialdehyde (MDA) was studied with the method developed by Wasowicz et al[6]. Malondialdehyde was condensed with two equivalents of thiobarbituric acid to give a fluorescent red derivative that could be assayed spectrophotometrically at λex = 525 and λem = 547 nm.

Table I. Study procedure.

Groups	Rats per group	Repeat time	Total rat per group
Group 1: Control **[Group C]**	2	1	2
Group 2: Contrast agent **[Group O]**	5	1	5
Group 3: Contrast agent + NAC **[Group N]**	5	1	5
Group 4: Diclofenac **[Group D]**	5	1	5
Group 5: Diclofenac + Misoprostol **[Group M]**	5	1	5
		Total rat count	**22**

Table II. Drugs and doses.

Drugs	Dosage	Administration way	Volume	Repeat time	Effect time
Radyoopaque iodinated contrast agent (Iohexol)	3 mg/kg	iv	1 cc/kg	1 time 3rd day	24 hours
N-acetylcysteine	200 mg/kg/day	oral	-	Daily Water	24 hours
Diclofenac sodium	2 mg/kg/day	oral	-	Bid	12 hours
Misoprostol	200 µg/kg/day	oral	-	Bid	12 hours

Total SH was studied with the method developed by Sedlak and Lindsay[7]. This method is based on Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid) or DTNB) and SH groups react forming TBN-SH (5 thio-2-nitrobenzoic acid) quantified in a spectrophotometer by measuring the absorbance of visible light at 412 nm.

Protein carbonyl was studied with the method developed by Reznick and Packer[8]. 2.4-Dinitrophenylhydrazine can be used to qualitatively detect the carbonyl functionality of a ketone or aldehyde functional group. A positive test is signalled by a yellow, orange or red precipitate, known as a dinitrophenylhydrazone.

Statistical Analysis

Values were evaluated with Statistical Package for the Social Sciences for Windows 10.0 (SPSS Inc., Chicago, IL, USA). Differences between groups were evaluated with Kruskal Wallis, Fisher Exact test and Mann Whitney U tests. In all tests, a value of $p < 0.05$ was accepted as statistically significant.

Results

During the follow-up no rats died. The rats were sacrified on the 7th day after drug administration and biochemical and histopathological examinations were made. Macroscopically edema was significantly less only in the misoprostol group. The right kidneys of all the rats were hydronephrotic.

Pathological Results

In the contrast agent + NAC obstructed group (Group NO) hemorrhage, chronic inflammation, obstructive dilatation and fatty degeneration was significantly less compared to the contrast agent obstructed group (Group OO) ($p < 0.05$). No significant difference was seen in respect of tubule epithelium damage between the two groups ($p > 0.05$). There was no significant difference between the normal kidneys ($p > 0.05$).

Between the diclofenac group (Group D) and the diclofenac + misoprostol group (Group M) no significant differences were detected in hemorrhage, chronic inflammation, obstructive dilatation, fatty degeneration and tubule epithelium damage in obstructed and normal kidneys ($p > 0.05$) (Tables III, IV).

In the contrast agent + NAC obstructed group (Group NO) the antioxidant SH level was significantly higher compared to the contrast agent obstructed group (Group OO) ($p < 0.05$). No significant difference was seen in MDA levels between the two groups ($p > 0.05$). In the contrast agent + NAC obstructed group (Group NO) carbonyl group was significantly less compared to the contrast agent obstructed group (Group OO) ($p < 0.05$). In normal kidneys no significant difference was seen ($p > 0.05$).

Between the diclofenac group (Group D) and diclofenac + misoprostol group (Group M) no significant difference was seen in SH, MDA and carbonyl levels in obstructed and normal kidneys ($p > 0.05$) (Tables V, VI).

Discussion

The administration of radiographic contrast agents often results in an acute reduction in renal function[9,10]. This reduction may cause substantial morbidity and mortality during hospitalization, which can lead to chronic end-stage renal disease[11,12]. Contrast agents reduce renal function by altering renal hemodynamics, by reactive oxygen species (ROS) and by exerting direct toxic effects on tubular epithelial cells[13,14]. Prophylactic oral administration NAC is known to reduce the incidence of acute contrast agent induced reductions in renal function[15].

There are various studies[16,17] in literature about protection against ureteral obstruction pathology.

Table III. Pathological results.

Groups	Group Name	Hemorrhage	Chronic inflammation	Obstructive dilatation	Fatty degeneration	Tubule epithelium damage
Group 1: Control **(Group C)**	Normal **CN**	-	-	-	-	-
	Obstructed **CO**	-	-	**+1*** **[2]**†	-	-
Group 2: Contrast agent **(Group O)**	Normal **ON**	**+1[2]**	**+1[2]**	-	**+1[2]** **+2[1]**	-
	Obstructed **OO**	**+1[4]** **+2[1]**	**+1[2]** **+2[3]**	**+2[4]**	**+1[5]**	-
Group 3: Contrast agent + NAC **[Group N]**	Normal **NN**	**+1[1]** **+2[2]**	-	-	-	**+2[1]** **+3[1]**
	Obstructed **NO**	**+1[1]**	**+1[1]**	**+1[2]**	**+1[1]**	-
Group 4: Diclofenac **(Group D)**	Normal **DN**	**+1[1]** **+2[2]**	**+1[1]**	-	**+2[2]**	**+1[2]** **+2[1]**
	Obstructed **DO**	**+1[3]**	**+1[3]** **+2[1]**	**+1[1]** **+2[1]**	**+1[2]** **+2[1]**	**+2[1]**
Group 5: Diclofenac + Misoprostol **(Group M)**	Normal **MN**	**+1[3]**	**+1[1]**	- **+2[3]**	**+1[1]**	
	Obstructed **MO**	**+1[1]**	**+1[3]** **+2[1]**	**+1[2]** **+2[2]** **+3[1]**	**+2[1]**	**-**

*+1 small changes, +2 average changes, +3 heavy changes. †In square brackets [Number of rats changes seen].

Table IV. Comparisons between pathological results (*p* values).

Groups	Hemorrhage	Chronic inflammation	Obstructive dilatation	Fatty degeneration	Tubule epithelium damage
Normal sides Contrast agent **(Group O)** Contrast agent + NAC **(Group N)**	0,307	0,134	1,000	1,000	1,000
Obstructed sides Contrast agent **(Group O)** Contrast agent + NAC **(Group N)**	**0,015**	**0,041**	**0,044**	**0,014**	1,000
Normal sides Diclofenac **(Group D)** Diclofenac + Misoprostol **(Group M)**	0,502	1,000	1,000	0,356	0,053
Obtructed sides Diclofenac **(Group D)** Diclofenac + Misoprostol **(Group M)**	0,221	1,000	0,065	0,343	0,317

Table V. Biochemical results. Average values of related biochemical study of each group.

Groups	Group name	Total SH µmol/mg protein	MDA nmol/mg protein	Carbonyl group nmol/mg protein
Group 1:				
Control **(Group C)**	Normal **CN**	3,7	54,1	1,52
	Obstructed **CO**	2,05	34,8	4,87
Group 2:				
Contrast agent **(Group O)**	Normal **ON**	4,05	57,2	3,50
	ObstructedOO	2,26	36,1	6,03
Group 3:				
Contrast agent + NAC **(Group N)**	Normal **NN**	3,80	82,1	4,02
	Obstructed **NO**	3,59	49,4	4,90
Group 4:				
Diclofenac **(Group D)**	Normal **DN**	3,89	65,0	3,09
	Obstructed **DO**	2,35	38,6	9,31
Group 5:				
Diclofenac + Misoprostol **(Group M)**	Normal **MN**	3,93	62,4	3,10
	Obstructed **MO**	2,36	32,7	7,29

Table VI. Comparisons between biochemical results (*p* values).

Group name	Total SH µmol/mg protein	MDA nmol/mg protein	Carbonyl group nmol/mg protein
Normal sides Contrast agent **(Group O)** Contrast agent + NAC **(Group N)**	0,564	**0,003**	0,697
Obstructed sides Contrast agent **(Group O)** Contrast agent + NAC **(Group N)**	**0,035**	**0,033**	**0,029**
Normal sides Diclofenac **(Group D)** Diclofenac + Misoprostol **(Group M)**	0,904	0,822	0,983
Obtructed sides Diclofenac **(Group D)** Diclofenac + Misoprostol **(Group M)**	0,977	0,513	0,410

Angiotensin antagonism is the most studied approach due to the clear link between angiotensin and renal injury and the availability of ACE inhibitors and angiotensin receptor blockers. Wamsley-Davis et al[18] administered the ACE inhibitor, enalapril, the AT1 antagonists losartan or candesartan, for up to 52 days to male rats with UUO. Candesartan inhibited the rise in JNK1 activity, losartan attenuated it, and enalapril did not affect it. Candesartan also reduced SMAD2 protein activation while attenuating the chronic tubulointerstitial fibrotic injury in obstructed kidneys and preserved renal mass. Trachtman et al[19] examined spironolactone in a rat model of unilateral ureteral obstruction (UUO). One week of obstruction produced minimal parenchymal damage, 2 weeks of obstruction produced renal fibrosis, which was significantly reduced by administration of the aldosterone antagonist spironolactone, without raising serum potassium or aldosterone concentrations.

Misoprostol is a prostaglandin E1 analogue widely used for off-label indications such as induction of labor in postdated pregnancy. Prostaglandin E1 and prostaglandin E2 are able to inhibit the transcription of endothelin. PGE1 also has cytoprotective effects[20]. More than 20 randomized controlled trials have assessed the efficacy of misop-

rostol in preventing NSAID-induced gastric ulcers[21]. Misoprostol works against drug-induced renal damage, interstitial cystitis, lupus nephritis, and hepatorenal syndrome[22]. It synergizes anti-inflammatory and the analgesic effects of diclofenac and has been administered to treat trigeminal neuralgic pain. In MEDIC Study (Misoprostol effects on diclofenac-induced cardiorenal changes in salt-sensitive patients with hypertension) co-administration of misoprostol with diclofenac attenuated the blood pressure elevation and renal vasoconstrictive effects of diclofenac and was well tolerated[23]. There is evidence supporting the role of prostaglandin involvement. Frokier and Sorensen[24] demonstrated an increase in PGE2 excretion in the urine from the contralateral kidney after UUO. In addition, studies[22,25,26] have shown that the increase in PGE2 and the vasodilation of the obstructed kidney could be blocked by indomethacin, a prostaglandin synthesis inhibitör.

In one study[28] misoprostol was found to be not effective on the renal function of rheumatoid arthritis patients treated with diclofenac. No clinically important interactions between misoprostol and NSAID were observed[29]. It is also known[30-32] that a combination product is available in many countries for protection from gastric ulcers. NSAIDs and narcotic analgesics are now most commonly used to treat pain associated with acute renal colic. Diclofenac is one of the most widely-used agents to reduce the pain. Although diclofenac can affect renal function in patients with already reduced function, it has no effect in patients with normal kidney function[33].

With ureteral obstruction, interstitial edema, widening of Bowman space, tubular basement membrane thickening, cell fattening, and cytoplasmic hyalinization have been demonstrated. Papillary tip necrosis, regional tubular destruction, and inflammatory cell response have been noted at 12 days[34]. Interstitial fibrosis and thickening of the tubular basement membranes were reported at 16 days after obstruction in a mouse model[35]. The inner cortex demonstrated severe tubular loss, proliferation of fibroblasts, and collagen deposition 3 weeks after obstruction in the porcine kidney. Cortical thinning and development of glomerular crescents were present at the 3 to 4 week interval in this model[32].

In the current work, the histopathological examination of rat kidneys showed that even the non-obstructed kidneys were affected by the drugs. NAC was seen to be protective against contrast agent side effects as expected, as has been shown in previous studies. Fewer pathological changes (hemorrhage, chronic inflammation, obstructive dilatation, fatty degeneration) were seen in the NAC group compared to all the other groups. The misoprostol diclofenac combination did not show any significant difference from diclofenac. Future studies could be designed over longer periods to show the protective effects of misoprostol against diclofenac.

Oxidative stress plays a significant role in the pathogenesis of UUO[36]. Yeh et al[37] demonstrated renal tubular apoptosis induced by oxidative stress and ER stress occurred in the UUO kidney. Many markers of oxidative stress are increased in UUO kidneys, such as the oxidatively damaged protein product Nε-carboxymethyl-lysine (CML), the modified amino acid 3-nitrotyrosine the marker of DNA oxidant damage, 8-hydroxy-2′-deoxyguanosine (8-OHdG) and lipid peroxidation markers such as malondialdehyde (MDA), 8-iso prostaglandin F2α (8-iPGF2α), and 4-HNE or 4-HHE[38-44].

Biochemical evaluation revealed that NAC is protective as a means of elevating antioxidant SH levels and decreasing carbonyl levels (showing less oxidative stress). MDA levels showed a reverse relationship. As MDA rises later compared to carbonyl levels and is less stable, this may make MDA less reliable. Studies over longer periods would be able to show MDA changes. No significant changes were seen biochemically between the diclofenac and diclofenac plus misoprostol groups. Misoprostol produced no positive effect against diclofenac toxicity although many studies have shown protective properties of this PGE1 analog.

New studies have been done recently regarding renal protection with pentoxyfilline after methotrexate treatment[45] and Ginkgo biloba extract against renal ischemia-reperfusion[46]. This agents may be studied for renal protection reasoned by ureteral obstruction and treatment of its medication.

Conclusions

We investigated misoprostol for protective effects against diclofenac in a UUO rat model in the light of the aforementioned studies whhich showed promising positive effects of this PGE1 analog. However, the study results showed no significant effect of misoprostol pathologically or biochemically. In fact in this research, NAC posi-

tive effects were seen over iv radiographic contrast agent as was known from previous rat investigations and clinical trials. Biochemical values are more prominent in shorter periods but pathological changes are more distinct after two or three weeks of obstruction. Different doses would show the effect of misoprostol more clearly. Further studies are required with different periods of obstruction and doses to validate the results of this study.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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