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ORIGINAL PAPER

The effect of diclofenac sodium on renal function in reversible unilateral ureteric obstruction

F. T. Hammad · L. Lubbad

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Abstract The objective of the study was to investigate the effect of diclofenac sodium, when administered similar to its use in clinical practice, 2 weeks following different periods of complete reversible unilateral ureteric obstruction in the rat using accurate indicators of renal function. Male Wistar rats underwent reversible left ureteric obstruction for 2 days [D2-untreated ($n = 8$) and D2-DS ($n = 8$) groups] and 5 days [D5-untreated ($n = 7$) and D5-DS ($n = 7$) groups]. D2-DS and D5-DS received intramuscular diclofenac sodium (3 mg/kg per day) during the time of obstruction, whereas D2-untreated and D5-untreated groups did not receive any treatment. The renal functions of both kidneys were studied 2 weeks following the reversal. The basal heart rate and mean arterial blood pressure were similar in D2-untreated and D2-DS and in D5-untreated and D5-DS groups. In the D2-untreated group, the GFR and RBF of the left obstructed kidney were 76 and 72% that of the right non-obstructed kidney [0.99 ± 0.06 vs. 1.30 ± 0.08 ($P < 0.05$) and 4.25 ± 0.33 vs. 5.92 ± 0.61 ($P < 0.05$), respectively]. The urine volume and urinary and fractional sodium excretion were not significantly different from the right kidney. Similar response was obtained in the D2-DS group. In the D5-untreated group, the GFR and RBF of the left kidney was 66 and 62% that of the right kidney (0.80 ± 0.12 vs. 1.21 ± 0.6, $P < 0.05$ and 3.79 ± 0.32 vs. 6.16 ± 0.59, $P < 0.05$; respectively). The tubular functions of both kidneys were similar. In the D5-DS group, there was no difference in GFR or RBF between both kidneys (1.02 ± 0.08 vs. 1.12 ± 0.3, $P = 0.24$ and 5.10 ± 1.25 vs. 6.46 ± 1.11, $P = 0.44$, respectively). Similarly, there was no difference in the tubular functions. Treatment with diclofenac sodium during a relatively long period of reversible unilateral ureteric obstruction, similar to its use in the management of ureteric colic, appears to ameliorate the alterations in the hemodynamic glomerular functions at least 2 weeks following the reversal of obstruction.

Keywords Ureteric obstruction · Diclofenac sodium · Renal function

Abbreviations

NSAIDs	Non-steroidal anti-inflammatory drugs
GFR	Glomerular filtration rate
RBF	Renal blood flow
UV	Urine volume
$U_{Na}V$	Urinary sodium excretion
FE_{Na}	Fractional excretion of sodium

Introduction

Ureteric obstruction is a relatively common clinical problem in humans, which may result in pain and renal damage [1]. The renal damage is due to the interaction of various factors and agents leading to alterations in hemodynamic and tubular renal function at the time of obstruction [2]. These agents include prostaglandins, the synthesis of which was found to be increased during the course of ureteric obstruction [3, 4].

Non-steroidal anti-inflammatory drugs (NSAIDs) have been used effectively in the management of ureteric colic

F. T. Hammad (✉) · L. Lubbad
Department of Surgery, Faculty of Medicine and Health Sciences, United Arab Emirates University, 17666, Al Ain, United Arab Emirates
e-mail: fayez@mail2doctor.com

[5, 6]. Indeed, currently, NSAIDs such as diclofenac sodium, are the most commonly used drugs in relieving obstruction-related mild to moderate ureteric colic. NSAIDs are a heterogeneous group of drugs, which act by blocking prostaglandin synthesis [7] and, hence, are expected to have a role in the functional alterations observed in the obstructed kidney. For instance, several animal studies have shown that NSAIDs had a potentially deleterious effect on the renal function as demonstrated by the decrease in the renal perfusion during the obstruction period [8–11]. This potentially toxic renal effect of NSAIDs was also supported by the clinical observation of renal function deterioration in some individuals such as elderly patients [12, 13]. However, this harmful effect of NSAIDs on the obstructed kidney was not observed universally and some studies have shown a beneficial effect of some NSAIDs in this condition. For example, in a rat model of a 24-h unilateral ureteric obstruction and contralateral nephrectomy, prostaglandins blockade by NSAIDs led to an improvement in renal functions [14]. In this study, the renal function was assessed by measuring serum creatinine, which was a rough estimate of renal function. Furthermore, the majority of these studies, including those which showed a deleterious effect of NSAIDs, used animal models with relatively short periods of ureteric obstruction and assessed the renal function either immediately or shortly after the release of the obstruction. Therefore, it is possible that the renal function, which was affected by the drug, could have returned to normal in the long term. Thus, the aim of this study was to investigate the effect of diclofenac sodium, when administered similar to its use in clinical practice, 2 weeks following different periods of complete unilateral ureteric obstruction in the rat using accurate indicators of renal function.

Methods

Studies were performed in male Wistar rats weighing 196–237 g at the time of ureteral obstruction. Rats were housed in standard cages and kept in a 12-h light–dark cycle at 20°C. They were fed a standard rat chow and had free access to water. Animals were fasted for 12 h before the experimental procedures, but had water ad libitum. The experimental protocol was approved by the local animal research ethics committee.

Ureteral occlusion operation and reversal

The following procedures were carried out under aseptic conditions. Each animal was anesthetized with ketamine hydrochloride (80 mg/kg, intraperitoneally, Pantex Holland B.V., Holland) and xylazine hydrochloride (8 mg/kg, intraperitoneally, Troy Laboratory PTY Limited, NSW, Australia). The left ureter was exposed via a midline abdominal incision and obstructed by placing a 3–4 mm length of bisected PVC tubing (0.58 mm internal diameter) around the left midureter as described previously [15]. The ureter was then occluded by constricting the tubing with a 4-0 silk suture. At the end, the wound was closed in layers.

The reversal of ureteric obstruction was performed using similar anesthesia as described previously. The left kidney and ureter were approached through the same incision. Using a dissecting microscope, the obstructing tube was identified and removed. Full release of the obstruction was confirmed by observation of a free flow of urine across the site of obstruction. The wound was then closed.

Diclofenac sodium administration

Diclofenac sodium (Berlin-Chemie, Germany) was administered at a dose of 3 mg/kg per day intramuscularly (thigh muscles) using an aseptic technique. Diclofenac sodium was given every 12 h, commencing just after the ureteric occlusion procedure, and the last dose was administered immediately after the reversal procedure.

Surgical procedure in the terminal experiment

Rats were fasted overnight, but had water ad libitum. Each animal was anesthetized with pentobarbital sodium BP (60–70 mg/kg, intraperitoneally; J M Loveridge plc, UK) and the trachea was cannulated. Following cannulation of a femoral vein with polyethylene tubing (PE-50), anesthesia was maintained by a continuous infusion of pentobarbital sodium (12.5 mg/kg/h) and a sustaining infusion of 0.9% saline was established at a rate of 50 μl/min using an infusion pump (Cole-Parmer, Vernon Hills, IL, USA). A femoral artery was cannulated with a tubing similar to that used in the femoral vein, and the tip of the cannula was positioned just below the level of the left renal artery. The cannula was connected to a pressure transducer (Memscap, Skoppum, Norway). The blood pressure signal was amplified using a bridge Amp (ADInstruments, Castle Hill, Australia), digitized using Power Lab 4/30 and Lab Chart version 6 software (ADInstruments, Australia), displayed on a computer screen. The arterial cannula was also used to obtain blood samples throughout the procedure as required. The left kidney was exposed through a midline abdominal incision and its upper ureter was cannulated with polyethylene tubing (PE-10) for the collection of urine into pre-weighed micro-capped tubes. The urine volume (UV) was determined gravimetrically. The right kidney underwent a similar procedure.

On completion of the surgery, a sustaining infusion of 0.9% saline was replaced by one composed of inulin (1.5% w/v) and para-aminohippuric acid (PAH) (0.2% w/v) in

0.9% saline. A priming dose of 2 ml of the same solution was infused over 2 min. Animals were allowed 2 h to equilibrate before being subjected to the experimental protocol.

Experimental protocol and assays

The experimental protocol consisted of two 15-min clearance periods. Arterial blood samples (0.4 ml) taken at the beginning and end of the clearance periods were immediately centrifuged. Plasma samples (125 µl) were frozen to be assayed later. The plasma was replaced by an equal volume of saline and the erythrocytes were resuspended by gentle vortexing and returned to the animal. The hematocrit was determined. Finally, the animals were euthanized with an overdose of barbiturate and the kidneys weighed.

The values of the measured variables including glomerular filtration rate (GFR), renal blood flow (RBF), UV, urinary sodium ($U_{Na}V$) and fractional excretion of sodium (FE_{Na}) were calculated as the average of the two clearance periods.

Experimental groups

Animals were divided into four groups:

1. D2-untreated ($n = 8$): rats that underwent left ureteric obstruction for 2 days.
2. D2-DS ($n = 8$): rats that underwent left ureteric obstruction for 2 days and received diclofenac sodium.
3. D5-untreated ($n = 7$): rats tht underwent left ureteric obstruction for 5 days.
4. D5-DS ($n = 7$): rats tht underwent left ureteric obstruction for 5 days and received diclofenac sodium.

The above four groups had their renal function measured 14–16 days after the reversal of the obstruction.

Statistical analysis

The collected data were entered into Microsoft Excel sheets and statistical analysis was performed using SPSS V16.0. Results are expressed as mean ± SEM. Comparison of the basal hemodynamic variables between groups was achieved using one-way factorial ANOVA and comparison between the right control and the left obstructed kidney in each group was performed by the *t* test. *P* value of less than 0.05 was considered to be statistically significant.

Results

As demonstrated in Table 1, which shows the mean arterial blood pressure and heart rate in the four groups, there was no difference in these variables between the two groups that underwent left ureteric obstruction for 2 days (D2-untreated and D2-DS) and also between the 5 days obstruction groups (D5-untreated and D5-DS).

Table 1 The basal mean arterial blood pressure and heart rate in the 2- and 5-day obstruction groups (please refer to text for group details)

	D2-untreated	D2-DS	D5-untreated	D5-DS
BP (mmHg)	108 ± 6	103 ± 7	112 ± 3	114 ± 5
HR (pulse/min)	370 ± 15	377 ± 8	390 ± 22	388 ± 24

Values represent mean ± SEM

In the D2-untreated group, the GFR of the left obstructed kidney was 76% that of the right non-obstructed kidney (0.99 ± 0.06 vs. 1.30 ± 0.08, $P < 0.05$) (Fig. 1). Similarly, the RBF of the left kidney was 72% that of the right kidney (4.25 ± 0.33 vs. 5.92 ± 0.61, $P < 0.05$). The tubular functional parameters of the left kidney were not significantly different from those in the right kidney [UV: 9.7 ± 1.2 vs. 12.3 ± 1.3 ($P = 0.16$); $U_{Na}V$: 2.5 ± 0.4 vs. 3.5 ± 0.5 ($P = 0.12$); FE_{Na}: 1.5 ± 0.2 vs. 1.6 ± 0.2 ($P = 0.76$)] (Fig. 2).

In the D2-DS group, the GFR of the left kidney was 71% of the right kidney (0.89 ± 0.06 vs. 1.26 ± 0.09, $P < 0.05$) (Fig. 1). The RBF of the left kidney was also

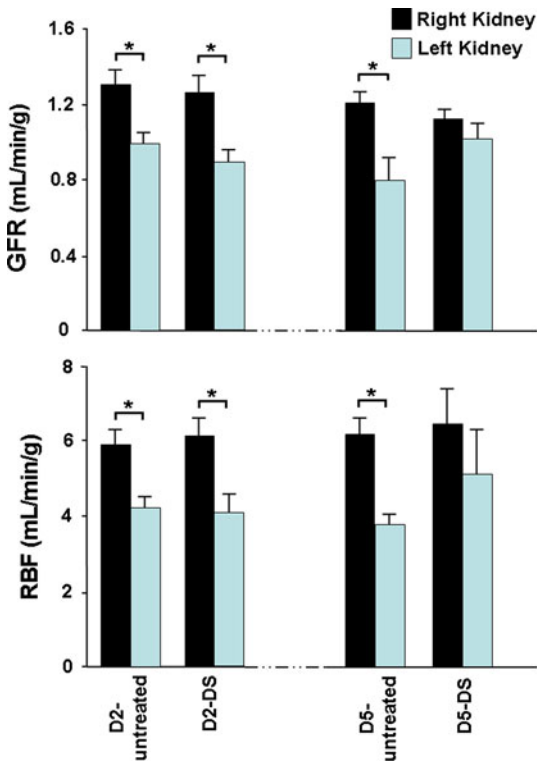

Fig. 1 The glomerular filtration rate (GFR) and renal blood flow in the 2- and 5-day obstruction groups (please refer to text for group details). Values represent mean ± SEM. *Asterisk* indicates statistical significance

significantly lower than that of the right kidney (67%, 4.07 ± 0.61 vs. 6.04 ± 0.54, $P < 0.05$). However, and similar to the D2-untreted group, there was no significant difference in the tubular function parameters between the two kidneys [UV: 9.5 ± 1.9 vs. 13.7 ± 1.5 ($P = 0.10$); $U_{Na}V$: 2.4 ± 0.5 vs. 3.8 ± 0.6 ($P = 0.11$); FE_{Na}: 1.7 ± 0.2 vs. 1.7 ± 0.2 ($P = 0.88$)].

In the D5-untreated group, the GFR of the left obstructed kidney was 66% that of the right kidney (0.80 ± 0.12 vs. 1.21 ± 0.6, $P < 0.05$) (Fig. 1). Similarly, the RBF of the left kidney was 62% that of the right kidney (3.79 ± 0.32 vs. 6.16 ± 0.59, $P < 0.05$). As demonstrated in Fig. 2 and similar to the 2-day obstruction group, the tubular functional parameters of the left obstructed kidney were not significantly different from those in the right kidney [UV: 11.7 ± 2.3 vs. 13.7 ± 2.8 ($P = 0.57$); $U_{Na}V$: 3.5 ± 0.7 vs. 4.3 ± 0.9 ($P = 0.47$); FE_{Na}: 2.5 ± 0.3 vs. 2.2 ± 0.5 ($P = 0.58$)].

In the D5-DS group, unlike the D2-DS and the D5-untreated groups, the GFR and RBF of the left kidney were not significantly different from those in the right kidney [1.02 ± 0.08 vs. 1.12 ± 0.03 ($P = 0.24$) and 5.10 ± 1.25 vs. 6.46 ± 1.11, $P = 0.44$] (Fig. 1). In addition, and similar to other groups, there was no significant difference in the tubular function parameters between the two kidneys [UV: 10.6 ± 2.3 vs. 12.0 ± 3.6 ($P = 0.74$); $U_{Na}V$: 2.5 ± 0.8 vs. 2.8 ± 1.1 ($P = 0.81$); FE_{Na}: 1.5 ± 0.4 vs. 1.6 ± 0.6 ($P = 0.92$)].

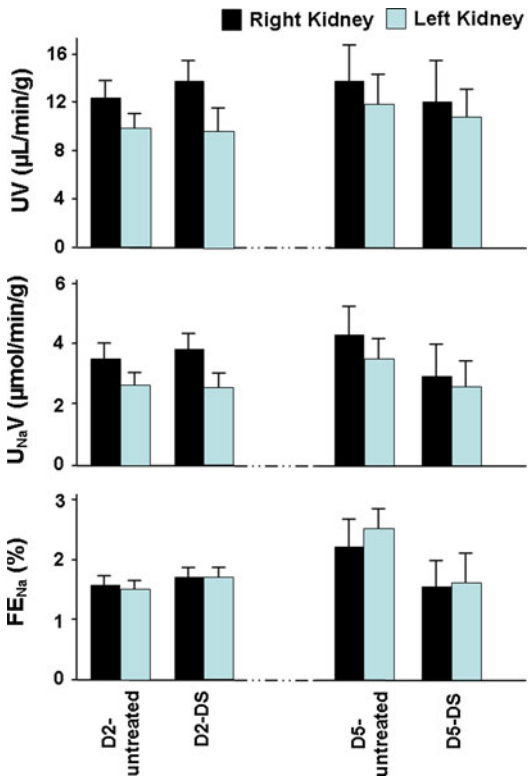

Fig. 2 The tubular functional parameters including UV, $U_{Na}V$ and FE_{Na} in the 2- and 5-day obstruction groups. Values represent mean ± SEM. There was no significant difference between the right and left kidney in any of the groups

Discussion

In clinical practice, it is well known that some patients with ureteric colic are prescribed diclofenac sodium or other types of NSAIDs for up to 6 weeks awaiting the passage of stones. However, the effect of this use on the renal function is not well established. On one hand, some animal studies have demonstrated that the blockade of prostaglandins resulted in an amelioration of the obstruction-caused alteration in the renal functional parameters, especially the hemodynamic functions [14]. Moreover, some NSAIDs were shown to decrease the tubular damage and interstitial fibrosis [16] caused by ureteric obstruction. On the other side, both animal [8–10] and human [12, 13] studies have shown that the exposure to NSAIDs especially when there is an underlying renal disease might result in deterioration in renal functions. This discrepancy is difficult to explain and could be due to several factors such as the type of NSAIDs used, the duration of ureteric obstruction, the time of measuring the renal function and the type of the renal functional parameter being measured. In this study, the administration of diclofenac sodium, one of the most commonly used NSAIDs, similar to its use in the clinical practice has resulted in an improvement in the GFR and RBF of the obstructed kidney 2 weeks following the reversal of 5-day obstruction.

In this study, we failed to prove any beneficial effect in the 2 days obstruction group. The exact reason is difficult to ascertain from the current results, but could possibly be due to the fact that the injury inflicted by the 2-day obstruction was less severe than the 5-day obstruction to show any significant benefit of diclofenac sodium. This was demonstrated by the higher drop in GFR in the 5-day compared to the 2-day obstruction groups (34 vs. 24%).

Two weeks following the reversal of ureteric obstruction of 2 and 5 days duration, the main alteration was in the glomerular hemodynamic functions as demonstrated by the decline in the GFR and RBF compared to the control kidney. The alterations in the tubular functions were insignificant as demonstrated by the similarities of the FE_{Na} between the obstructed and non-obstructed kidneys. However, it has been demonstrated that ureteric obstruction, especially for long periods, results in an alterations in the tubular function, which may take some time to recover [17]. Therefore, it appears from the data in this study that the tubular function of the obstructed kidney must have improved by 2 weeks post-reversal of the obstruction.

Furthermore, it is not known if diclofenac sodium would have affected the initial alterations in the tubular function, although there is evidence from other studies in support of this effect [17].

Similar to the tubular functions, hemodynamic renal functions including GFR and RBF were shown to improve over time following reversible unilateral ureteric obstruction, and might return to basal levels especially with short periods of obstruction. For instance, in a reversible 24-h unilateral ureteric obstruction in the rat, the basic renal functional parameters including GFR and BBF returned to pre-obstruction levels within 2 weeks of the reversal [15, 17]. From the current study, it is not known if the GFR and RBF of the obstructed kidney would have improved over time beyond 2 weeks and ultimately returned to normal. Should this be the case, then diclofenac sodium would not have been expected to show any beneficial effect in the longer term when these functions would have returned to basal levels anyway and further research is required to address this issue.

In the current study, the subcutaneous route was used to administer diclofenac sodium because it is usually a more reliable method compared to the oral intake. In addition, we used a similar dose to that in other studies [18]. Therefore, it appears unlikely that the drug was not absorbed into the system. This was supported by the improvement in the renal functions observed in the left kidney of the D5-DS group, which indicated that the drug was absorbed and had exerted its effect.

This study has some limitations, and caution should be taken in extrapolating the results to different experimental models and clinical scenarios. For instance, care should taken on applying the current results to individuals with any underlying degree of renal impairment, with different periods of ureteric obstructions or in cases of partial ureteric obstruction that mimics the relatively common clinical scenario of partially obstructing ureteric calculus. Care should also be taken on applying the current findings to other types of NSAIDs. In addition, the creation of ureteric occlusion in this study was performed by ligating a bisected PVC tubing around the ureter (extramural obstruction). Whether the results obtained in this study would also apply to the scenario of ureteric obstruction by the intramural urinary calculus is difficult to ascertain and more studies are required to clarify this point.

Obviously, other factors that affect the final outcome following ureteric obstruction include the presence of urinary infection, which is not uncommon in the clinical scenario of obstructing ureteric stones. This was not measured in this study, but all the surgical procedures were performed under completely aseptic technique and certainly none of the rats included in the study showed any signs of infection or distress during the obstruction or post-reversal. Therefore, the role of diclofenac sodium in ureteric obstruction associated with urinary infection requires further clarification.

In conclusion, the administration of diclofenac sodium during a relatively long period of unilateral ureteric obstruction, similar to its use in the management of ureteric colic, appears to ameliorate the alterations in the hemodynamic glomerular functions including the GFR and the RBF at least 2 weeks following the reversal of obstruction.

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