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Original Article

Induction of Chronic Renal Failure in Goats Using Cisplatin: A New Animal Model

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ABSTRACT

Cisplatin was administered at the dose rate of 30 mg m^{-2} daily intravenously consecutive for 7 days in goats. Blood samples (2 ml) were collected from each goat at '0' hr and then at weekly interval and centrifuged immediately at 3000 rpm for 20 min to separate plasma, which were used for estimation of blood urea nitrogen (BUN), plasma creatinine (CRT), gamma glutamyltransferase (γGT), and glomerular filtration rate (GFR). Total volume of urine of each goat was recorded, and 5 ml of urine samples were collected for estimation of GFR. Blood urea nitrogen started to increase significantly from 7 days post-dosing and achieved a peak on day 14. Higher values persisted up to 91 days. Plasma creatinine level was significantly higher in all samples on day 7 onwards, and it was maintained up to day 91 post-dosing compared to control samples ('0' day) whilst GFR declined significantly from day 7 and attained a minimum values on day 70. GFR was almost <60% up to 91 days. The signs like emaciation, loss of body weight, and oliguria were observed. The values of all 4 biomarkers showed a chronic renal failure in goats.

Key words: Blood urea nitrogen, chronic renal failure, cisplatin, GGT, glomerular filtration rate, goat, plasm creatinine

INTRODUCTION

Cisplatin, an anti-neoplastic heavy metal complex, is one of the most active drugs used in the treatment of tumors including advanced ovarian and small cell lung cancer. It is also used as a treatment for testicular, ovarian, bladder, head, lung, and neck cancer in combination with other drugs including, cyclophosphamide, bleomycin, and etoposide. The dose-limiting factor in cisplatin treatment is its well-documented nephrotoxicity and neurotoxicity, the nephrotoxicity resulting in necrosis of proximal tubule (PCT) and collecting ducts cells.[1] Because acute kidney disease may progress to a chronic condition, any cause of acute kidney disease is also a possible cause of chronic kidney disease.[2] Induction of acute renal failure in goats using uranyl nitrate has been reported;[3,4,5] but induction of chronic renal failure in goats by any chemical agents is scarily available though cisplatin, an anti-cancer agent, has been used in humans[6] and also caused acute renal failure in rats.[7,8] Chronic renal failure often the consequence of slow, insidious destruction of renal parenchyma. Progressive destruction of renal tissues has been presumed to occur because of persistence of the initiating cause. Many abnormalities are only apparent when GFR is reduced to less than one-third of normal.[9]

Chronic renal failure is one of the major problems in goats. It is very difficult to find the efficacy or bioavailability of drug in experimental goats. No literatures are available to in due chronic renal failure in goats.

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Keeping all these in views, the present research work was undertaken to establish chronic renal failure model after intravenous administration of cisplatin in goats, which will be utilized for efficacy or bioavailability study of drugs.

MATERIALS AND METHODS

Test drugs

Cisplatin, used for the induction of chronic renal failure, was purchased as trade name KEMOPLAT™ (1 mg ml^{-1}, 50 ml vial) injection, marketed by Dabur Pharma Ltd. Village - kishanpura, P.O. Guru Majra, Tehsil - Nalagarh, Distt. Solan, (H.P.) - 174 101, INDIA. All the chemicals/ reagents in this study were obtained from E.Merck (India), Sigma Chemicals Co. (USA), Rankem (India) and Sisco Research Laboratories Ltd (India).

Experimental animal

Clinically healthy nine Black Bengal male and female (Nulliparous and non-pregnant) adult goats (1-2 years age) weighing between 10-12 kg were used in this experiment. They were kept individually in the custom-made stainless steel metabolic cages (size 48′ × 48′ × 36′). The animals were stalled-fed, and water was provided ad libitum. The goat feed was supplied by EPIC, Kalyani, West Bengal. The temperature of the animal room was maintained at 25 ± 2°C and provided with artificial lighting facilities.

Before starting the experiment, the animals were de-wormed once with a mixture of levamisol hydrochloride I.P. (1.5% w/v) and oxyclozanide I.P. (Vet) (3.0% w/v suspension FLUZAN™, Jeps Pharma (p) Ltd.), C-207, Naraina Industrial Area, phase-1, New Delhi-110 028) at the total dose rate of 1 ml/2 kg b.w. After 21 day of de-worming, the animals were acclimatized in experimental environment for 7 days. The animals were monitored throughout the experiment period. The study was approved by the Institutional Animal Ethics Committee.

Induction of chronic renal failure

Nine goats were selected for the study and were divided into 3 groups (I, II and III) each containing 3 animals.

Cisplatin was administered to each goat of all 3 groups (I, II and III) at 3 different dosage levels. Group I goats received cisplatin at 12 mg m^{-2} intravenously for consecutive 5 days. Cisplatin was administered to the goats of group II @ 20 mg m^{-2} at weekly interval up to 5 dosages intravenously, whilst group III goats received cisplatin at 30 mg m^{-2} intravenously for 7 successive days.

Collection of samples

Blood

Blood samples (5 ml) were collected in heparinized test tubes from jugular veins of each goat of all 3 groups and were centrifuged immediately at 3000 rpm for 20 min for separation of plasma, which were transferred to scintillations vials to store at –20°C until further use for the estimation of plasma creatinine, plasma urea nitrogen, and glomerular filtration rate.

Urine

Total excreted urine volume from each goat of all groups were measured and recorded throughout the experimental period. Urine samples (5 ml) were collected and centrifuged at 2000 rpm for 20 min. Upper layer was collected, and sediment was discarded. Collected urine samples were cleaned up thrice with hexane in separatory funnel and stored at –20°C till further use for GFR.

Confirmation of chronic renal failure

Induction of chronic renal failure after administration of cisplatin to each goat was confirmed by the following biomarkers:

i. Blood urea nitrogen (BUN)
ii. Plasma creatinine level (CRT)
iii. Gamma glutamyltransferase (γGT) in urine
iv. Glomerular Filtration Rate (GFR)
v. Ultrasound trucut biopsy of kidney.

Estimation of blood urea nitrogen

Blood urea nitrogen was estimated by using diagnostic kit (M/S Span Diagnostics Ltd., Surat, INDIA) as per DAM method described by Coulambe *et al*.[10]

Estimation of plasma creatinine level

Plasma and urine creatinine level was estimated by using diagnostic kit (M/S Span Diagnostics Ltd., Surat, INDIA) as per Alkaline Picrate Method described by Varley *et al*.[11]

Estimation of gamma glutamyltransferase

Urine gamma glutamyltransferase enzyme was analyzed by measuring the release of p-nitroanilide method described by Meister *et al*.[12]

Determination of glomerular filtration rate

Glomerular filtration rate was determined by creatinine clearance (Cl_{crt}) method described by Cockcroft-Gault *et al*.[13]

C.4.4. Histopathology

Histopathology of kidney tissue was performed by ultrasound trucut biopsy technique.

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Statistical analysis

All parameters were determined for each animal individually, and the mean and standard error (SE) were calculated. Mean values, SE, and analysis of variance (ANOVA) of the tabulated data were calculated where applicable by using standard formulas.[14]

RESULTS AND DISCUSSIONS

Induction of chronic renal failure by cisplatin

Biomarkers

Blood urea nitrogen

Mean values with SE of blood urea nitrogen level in goats following consecutive daily intravenous administration of cisplatin for 7 days at 30 mg m^{-2} have been depicted in Table 1 and Figure 1. Blood urea nitrogen level in goats before administration of cisplatin ('0' day) was 7.49 ± 0.34 mg dl^{-1}, which started to increase from day 7 (21.27 ± 3.42 mg dl^{-1}), peaked on day 14 (30.67 ± 0.32 mg dl^{-1}) and maintained a plateau till 35 day. BUN level was significantly ($P < 0.05$) higher in all the samples from day 7 onwards and up to day 91 post-dosing of cisplatin compared to 0 day [Table 1].

Plasma creatinine

Mean values with SE of plasma creatinine level in goats following consecutive daily intravenous administration of cisplatin for 7 days at 30 mg/m^2 have been presented in Table 1 and Figure 2. Plasma creatinine (CRT) level in goats before administration of cisplatin (0 day) was 0.88 ± 0.04 mg dl^{-1}. The value was observed to increase from day 7 (1.18 ± 0.06 mg dl^{-1}) and reached a maximum concentration of 1.94 ± 0.19 mg dl^{-1} on day 14. The CRT level was significantly ($P < 0.05$) higher in all the samples collected from 1 day onwards and up to day 91 post-dosing of cisplatin compared to control sample of day 0 [Table 1].

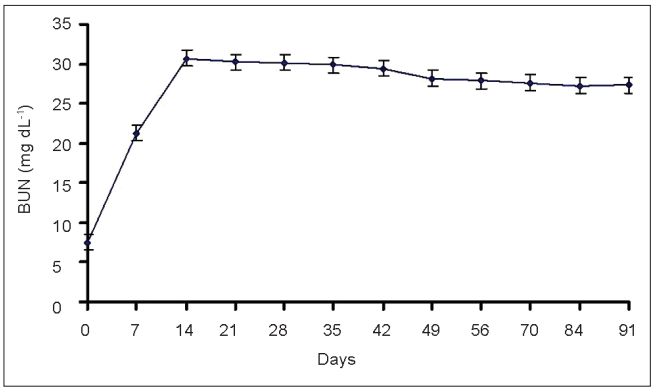


Figure 1: Effect of cisplatin on blood urea nitrogen (mg dl^{-1}) level in goats following consecutive daily intravenous administration for 7 days at 30 mg m^{-2}

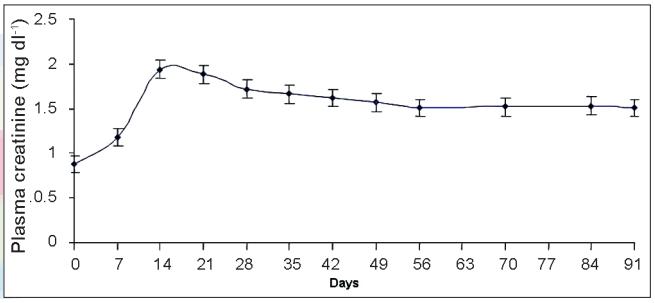


Figure 2: Effect of cisplatin on plasma creatinine (mg dl^{-1}) level in goats following consecutive daily intravenous administration for 7 days at 30 mg m^{-2}

Table 1: Effect of cisplatin on blood urea nitrogen (mg dl^{-1}), plasma creatinine (mg dl^{-1}) level, urine gamma glutamyl transpeptidase (µmole p-nitroanilide formed min^{-1} ml^{-1}) and glomerular filtration rate (ml min^{-1} kg^{-1}) in goats following consecutive daily intravenous administration for 7 days at 30 mg m^{-2}

Days	Parameters			
	Blood urea nitrogen (mg dl^{-1})	Plasma creatinine (mg dl^{-1})	Urine gamma lutamyl transpeptidase (µmole p-nitroanilide formed min^{-1} ml^{-1})	Glomerular filtration rate (ml min^{-1} kg^{-1})
0	7.49±0.34[a]	0.88±0.04[a]	7.81±0.84[a]	1.84±0.18[a]
7	21.27±3.42[b]	1.18±0.06[b]	12.40±0.71[b]	1.42±0.15[b]
14	30.67±0.32[b]	1.94±0.19[b]	13.84±0.33[b]	0.99±0.05[b]
21	30.28±0.13[b]	1.88±0.06[b]	15.66±0.61[b]	0.83±0.10[b]
28	30.19±0.17[b]	1.72±0.10[b]	19.01±0.68[b]	0.75±0.14[b]
35	29.86±0.09[b]	1.66±0.14[b]	19.58±0.54[b]	0.69±0.03[b]
42	29.39±0.11[b]	1.62±0.04[b]	18.67±0.35[b]	0.64±0.10[b]
49	28.15±0.04[b]	1.57±0.23[b]	16.99±0.38[b]	0.58±0.19[b]
56	27.90±0.16[b]	1.51±0.21[b]	16.57±0.29[b]	0.59±0.17[b]
70	27.59±0.21[b]	1.52±0.01[b]	15.27±0.74[b]	0.55±0.03[b]
84	27.28±0.14[b]	1.53±0.12[b]	14.66±0.74[b]	0.58±0.11[b]
91	27.31±0.26[b]	1.51±0.20[b]	12.97±0.63[b]	0.56±0.07[b]

Mean value with dissimilar superscript (ab) in column vary significantly ($P<0.05$)

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Urine gamma glutamyltransferase

Mean values with SE of gamma glutamyl transpferasase activity in goats following consecutive daily intravenous administration of cisplatin for 7 days at 30 mg m^{-2} have been described in Table 1 and Figure 3. The γGT activity in goats before administration of cisplatin (0 day) was 7.81 ± 0.84 µmole p-nitroanilide formed min^{-1} ml^{-1}. The values were recorded to be increased from day 2 and showed a maximum concentration on day 14 (19.58 ± 0.54 µmol p-nitroanilide formed min^{-1} ml^{-1}). The γGT was significantly ($P < 0.05$) higher in all samples of day 5 onwards and up to 91 days (12.97 ± 0.63 µmole p-nitroanilide formed min^{-1} ml^{-1}) of cisplatin treatment compared to control sample (day 0).

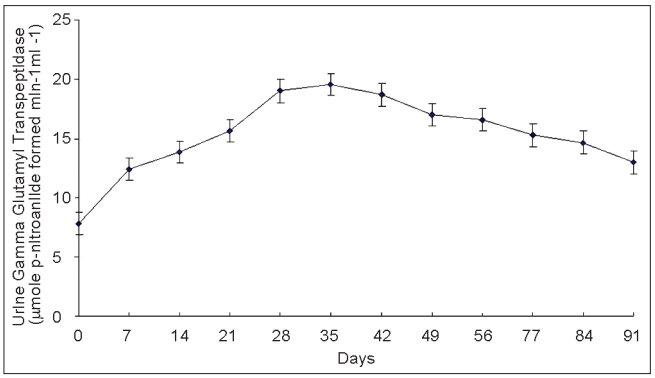


Figure 3: Effect of cisplatin on urine gamma glutamyl transpeptidase (µmole p-nitroanilide formed min^{-1} ml^{-1}) level in goats, following consecutive daily intravenous administration for 7 days at 30 mg m^{-2}

Glomerular filtration rate

Mean values with SE of glomerular filtration rate in goats following consecutive daily intravenous administration of cisplatin for 7 days at 30 mg m^{-2} have been incorporated in Table 1 and Figure 4. Glomerular filtration rate (GFR) in goats before administration of cisplatin (0 day) was 1.84 ± 0.18 ml min^{-1} kg^{-1}), which was gradually declined significantly ($P < 0.05$) from day 7 (1.42 ± 0.15 ml min^{-1} kg^{-1}) to day 91 of cisplatin treatment compared to control sample day 0 [Table 1].

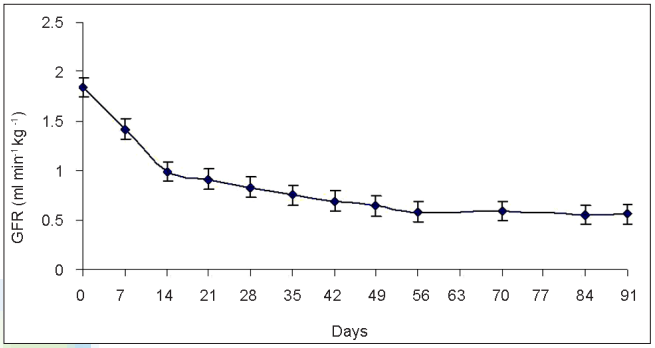


Figure 4: Effect of cisplatin on glomerular filtration rate (ml min^{-1} kg^{-1}) level in goats following consecutive daily intravenous administration for 7 days at 30 mg m^{-2}

Histopathological study

Section of kidney of animal after administration of cisplatin at 30 mg m^{-2} intravenously shows partial atrophic changes of glomeruli, degenerative changes in most of the tubules with necrotic changes (Plate II) compared to Plate I. On other hand, increased Bowman's space and no vascular damage were observed. The proximal and distal convoluted tubules showed partial degeneration and loss of epithelia (Plate II) whilst Plate I shows the section of kidney of animal of healthy normal architecture of parenchyma of kidney [Figure 5].

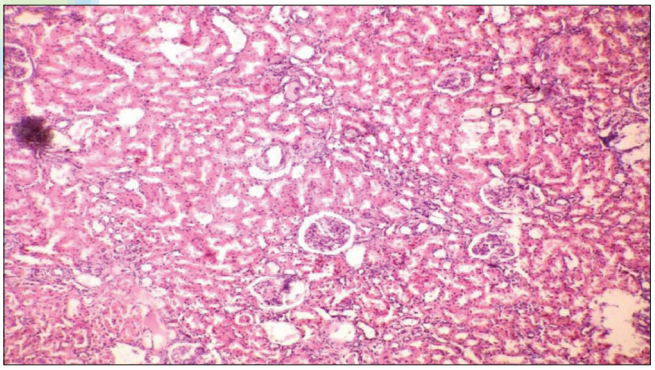


Figure 5: Section of kidney of animals group II (CRF) showing partial atrophic changes of glomeruli, degenerative changes in tubules including PCT and DCT (H and E, ×40)

Zhou *et al.*[8] found a significant increased in BUN and plasma creatinine values and renal morphological changes including extensive tubular damage in acute renal failure rats after intravenous administration of cisplatin (6 mg kg^{-1} b.w.). Asna *et al.*[15] observed a significant increase of blood urea nitrogen, higher BUN to plasma CRT ratio, reduced hemoglobin level and platelets count following consecutive 5 days intra-peritoneal administration of cisplatin in male mice leading to nephrotoxicity in mice. Dutta *et al.*[4] found that administration of uranyl nitrate at rate of 0.75 mg kg^{-1} in pyrogen-free distilled water (10 ml) intravenously to left jugular vein of goat for consecutive 5 days caused kidney damage. To ascertain desired level of damage of kidney, BUN and CRT levels were monitored every 24 hrs starting from 0 hr. The dose level significantly increased the BUN level from 24 hr onwards, and maximum increase was recorded at 96 hr. Plasma creatinine level of goats increased from 24 hr onwards. Maximum increase was recorded at 96 hr post-dosing.

The blood urea nitrogen (BUN) and plasma creatinine (CRT) are the commonly used biomarkers to study intensity of renal failure due to the fact that it is released in to the plasma at relatively constant rate in healthy state, which are freely filtered by the glomerulus and neither metabolized nor released by the kidney. The actual plasma creatinine may be increased or decreased, independent of changes in the glomerular filtration rate, by inhibiting or stimulating renal tubular secretion. glomerular filtration rate and renal gamma glutamyltransferase are the active biomarkers of chronic kidney diseases.[9,16] Cisplatin appears to enter cells

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by diffusion. The chloride atoms may be displaced directly by reaction with nucleophiles such as thiols; replacement of chloride by water yields a positively charged molecule and is probably responsible for formation of the activated species of the drug, which then reacts with nucleic acids and proteins, causing necrosis and damage to proximal tubule.[17] Development of necrotic and fibrotic lesion at the corticomedullary junction is observed due to increased blood urea nitrogen and plasma creatinine levels. Wainford *et al*.[17] studied the mechanism of cisplatin causing nephrotoxicity *in vivo* and *in vitro* model system. Nephrotoxicity was induced in rats (6 mg kg^{-1} cisplatin i.p.) and mice (10 mg kg^{-1} cisplatin i.p.). Cisplatin administration significantly elevated BUN and serum creatinine in male Sprague Dawley rats on day 5 post-treatment (BUN 28 ± 5 μmmol ml^{-1}; serum creatinine 108 ± 4 nmom ml^{-1}; $P < 0.05$) and in male mice day 4 post-treatment (BUN 21 ± 1 μmol ml^{-1}; serum creatinine 81 ± 5 nmol ml^{-1}; $P < 0.05$). Nephrotoxicity was confirmed by histological changes that showed significant damage to the proximal tubules of cisplatin versus saline vehicle-treated animals.

BUN and CRT have been significantly increased on day 1 and day 5, respectively, by cisplatin injection. Partial atrophic changes of glomeruli and degenerative changes in most of the tubules with necrotic changes were observed. No cast was seen. Stromal tissue appears unremarkable. On other hand, increased Bowman's space and no vascular damage were observed. The proximal and distal convoluted tubules showed partial degeneration and loss of epithelia. Chronic renal failure is developed due to irreversible chronic tubular necrosis by cisplatin injection without showing any symptoms of nephropathy in experimental goats. Arany *et al*.[18] showed that the major dose-limiting side-effect is nephrotoxicity, which evolves slowly and predictably after initial and repeated exposure. The kidney accumulates cisplatin to a higher degree than other organs perhaps via mediated transport. Functionally, reduced renal perfusion and a concentrating defect characterize its nephrotoxicity, whereas morphologically necrosis of the terminal portion of the proximal tubule and apoptosis predominantly in the distal nephron characterize its effects on cell fate. Daugaard *et al*.[6] studied the pathophysiological mechanisms involved in cisplatin-induced nephrotoxicity. Immediately after administration of cisplatin to human, renal blood flow and re-absorption rates decreased significantly. These data was confirmed in a micropunture study in rats. After administration of 20 mg cisplatin per m^{-2} for 5 days in humans, a small but significant decrease in GFR was observed. In the high-dose cisplatin group (40 mg m^{-2} for 5 days), a severe decrease in GFR was observed during treatment, and GFR remained decreased for up to 2 years after termination of treatment. The observation of an acute increase in N-acetyl-β-D-glucosamidase indicates a primary tubules effect of cisplatin in humans. These changes persist for at least 6 months after treatment.

Therefore, a dose of 30 mg m^{-2} of cisplatin for consecutive daily intravenous administration in goats may be used for induction of chronic renal failure of model, which will persist till 90 days.

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