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Exposure to imipenem/cilastatin causes nephrotoxicity and even urolithiasis in Wistar rats

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ABSTRACT

Imipenem/cilastatin is a broad-spectrum β-lactam antibiotic used to treat several bacterial infections. The present study was designed to validate the nephrotoxic effect of this drug in rats and to explore its potentional urolithiatic effect. Thirty two Wistar rats were randomly divided into four groups: three experimental groups treated with different imipenem/cilastatin dosages (30, 50 and 80 mg/kg/day) and a control group.The experimental groups were given intraperitoneal imipenem/cilastatin injections twice daily for 7 days, and the control group was given intraperitoneal vehicle NaCl 0.9% solution. Nephrotoxic effect of this antibiotic was assessed based on urine and plasma biochemistry, oxidative stress parameters, histopathological examination and infrared spectroscopy characterization. Imipenem/cilastatin administration resulted in alkaline urine, polyuria, crystalluria, raised plasma levels of urea, creatinine and uric acid, decreased contents of plasma gamma glutamyltranspeptidase and alkaline phosphatase, oxidative stress status, malpighian metaplasia as well as crystal deposition in kidneys and urinary tracts of Wistar rats. In addition, the precise nature of the calculi was identified, being formed by imipenem/cilastatin, thus confirming their iatrogenic origin. In conclusion, this study demonstrated through rat model that subacute exposure to imipenem/cilastatin may induce nephrotoxicity and increase the risk for developing kidney stones even at therapeutic dose levels in a dose-dependent manner.

1. Introduction

Urolithiasis is one of the main constraints to human health in the world, regardless of racial, cultural and geographical boundaries (Aggarwal et al., 2013; Panigrahi et al., 2016). Currently, the annual incidence of kidney stones is increasing leading to many severe health troubles such as hemorrhage, hydronephrosis, obstruction and infection in the urinary tract. Calcium oxalate is the most frequent component of calculi with a percentage greater than 80% of the analysed stones (Vyas et al., 2011). Lifestyles, diets, habits, obesity and hypertension seem to be the most common causes of urolithiasis (Obligado and Goldfarb, 2008). Regarding iatrogenic lithiasis induced directly or indirectly by the action of medical treatments, medication lithiasis is particularly a low prevalent type. Indeed, using infrared techniques, only a 2% incidence and a single triamterene calculus have been reported respectively by Jungers et al. (1989) and Hidalgo et al. (1983).

Imipenem/cilastatin is one of the broadly effective penems found to be nephrotoxic in previous reports (Tune, 1994, 1997). Generally, imipenem is used at doses ranging from 15 to 50 mg/kg for adults, in combination with cilastatin which is an inhibitor of renal dehydropeptidase (Wolff et al., 2009). Cilastatin prolongs the half-life of imipenem in the blood from 0.4 to 0.9 h (Sack et al., 1985) . According to the manufacturers, both of imipenem and cilastatin are not nephrotoxic in rats (Sack et al., 1985). On the other hand, in 1985, Sack et al. reported that both imipenem and imipenem/cilastatin in combination induced tubulotoxic effects in a dose-dependent manner when administered intraperitoneally to rats at 150 mg/kg over 7 days at dosage intervals of 12 h (Sack et al., 1985). In 1989, Topham et al. demonstrated that imipenem/cilastatin administration may lead to renal tubular necrosis in rabbits as well as in cynomolgus monkeys (Topham et al., 1989). This investigation was performed to elucidate the detailed mechanism of imipenem/cilastatin toxicity on the renal system by studying some biochemical parameters, antioxidant status and histopathological changes in the kidney of rats exposed to imipenem/cilastatin at two therapeutic doses and an overdose to have a better knowledge about the exact margin of toxicity of this antibiotic. In addition, the potential imipenem/cilastatin −induced urolithiasis was also examined using rats to establish the rat as an appropriate animal

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model. Since imipenem/cilastatin is one of the β-lactam antibiotics that share similar carriers in humans and animals (Ruiz-Carretero et al., 2004), the possibility of lithiasis may be taken into account in patients.

2. Materials and methods

2.1. Animals and treatment

The study protocol was performed in accordance with international guidelines for Care and use of laboratory animals (Council of European Communities, 1986) and approved by the Local Animal Care Commitee of Sfax University.

In this investigation, 32 adult male Wistar rats, weighing about 250–270 g purchased from the Central Pharmacy (SIPHAT, Tunis, Tunisia) were maintained for 1 week under standard laboratory conditions of temperature (23 ± 1 °C), relative humidity (70 ± 4%) and a 12 h light/12 h dark cycle. They were provided daily with standard pellet diet and water ad libitum. After the period of acclimatization, the animals were allocated into the following four groups each consisting of eight rats: Animals of the group I (Control group) were given a vehicle NaCl 0.9% solution by intraperitoneal way, daily, for 7 days. Animals of groups II, III and IV were received daily intraperitoneal injection of 30,50 and 80 mg/kg of imipenem/cilastatin respectively, (Golchin et al., 2013; Mimoz et al., 2000) for 7 successive days (2 injections each day) (Sack et al., 1985; Tahri et al., 2017).

Imipenem/cilastatin (1:1) is marketed under the trademark "SYNERGIC®" by UNIMED-TUNISIE laboratories. It is a powder for solution for injection containing 500 mg of imipenem and 500 mg of cilastatin combined in a pharmaceutical ampoule preparation. This drug was dissolved in a vehicle NaCl 0.9% solution (50 mg/ml) prior to use. The doses of 30 and 50 mg/kg were in the therapeutic level while 80 mg/kg was a high dose (Wolff et al., 2009; Tahri et al., 2017). Throughout the experimental period, all animals survived and their weights were monitored daily.

For a collection of 24 h urine samples, 12 h after the last dose, animals were kept in individual metabolic cages with free access to water and food. After determination of volume, pH, specific gravity and crystalluria, urine samples were stored at −20 °C for further analysis.

After 8 day of the experiment, all rats were anaesthetized with chloral hydrate and sacrificed by decapitation (Sefi et al., 2010). Blood was collected from the heart of each rat into heparincoated tubes and centrifuged at 1500 rpm for 15 minThe plasma samples were stored at −20 °C for biochemical assays.

Kidneys tissues were removed, separated from adipose tissue and weighed. The right kidneys were cut into small pieces and homogenized in ice-cold NaCl 0.9% solution ((w/v) = 10%) using Ultra Turrax tissue homogenizer. The homogenates were centrifuged at 3,000*g* for 10 min at 4 °C and the supernatant fluids were kept at −80 °C for the biochemical estimations. The left kidneys were fixed in 10% buffered formalin solution and embedded in paraffin for histological analysis.

2.2. Determination of volume, pH and crystalluria of urine

Volume and pH of urine were assessed immediately according to the method of Bahuguna et al. (2009). Specific gravity was measured as described by Liu et al. (2016) and crystalluria was determined by microscopic examination in order to identify urinary crystals.

2.3. Assessment of creatinine, urea, uric acid and creatinine clearance

The levels of creatinine, urea and uric acid in plasma and urine were assessed spectrophotometrically using commercial reagent Kits (Biomaghreb, Tunisia, Ref. 20151, 20143, 20095, respectively) according to the manufacturer's instructions. Creatinine clearance was calculated as reported by Charrel (1991).

2.4. Evaluation of plasma gamma glutamyltranspeptidase and alkaline phosphatase activities

Plasma gamma glutamyltranspeptidase and alkaline phosphatase activities were evaluated using diagnostic kits (Biomaghreb, Tunisia, Ref. 20022 and 20015, respectively) according to the manufacturer's instructions.

2.5. Assay of tissue biomarkers

2.5.1. Lipid peroxidation

Lipid peroxidation in kidney homogenates was evaluated based on the generation of thiobarbituric acid reactive substances and expressed as the extent of malondialdehyde production according to Latchoumycandane and Mathur (2002).

2.5.2. Enzymatic antioxidants

Superoxide dismutase activity was determined as described in the method of Sun et al. (1988), which was slightly modified by Durak et al. in 1993, and expressed as units per milligram of protein.

Tissue catalase activity was assessed following the method of (Aebi, 1984) and expressed in micromoles of H_2O_2 consumed per minute per milligram of protein at 25 °C.

Glutathione peroxidase activity was spectrophotometrically assayed by the method of Paglia and Valentine (1967). The enzyme activity was expressed as micromoles of GSH oxidized per minute per milligram of protein.

2.5.3. Protein oxidative markers

The concentration of advanced oxidation protein products, as a marker of oxidative modified proteins and also inflammation, was estimated by a spectrophotometric method described by Kayali et al. (2006). It was expressed as nmol/mg protein.

Protein carbonyl levels in kidney were determined according to the method of Reznick and Packer (1994). Values were expressed as nmol/mg protein.

2.5.4. Proteins concentration

The concentration of proteins was measured according to Bradford's method (1976), using bovine serum albumin as standard.

2.6. Histological analysis

The kidney tissues fixed in 10% buffered formalin solution, were dehydrated in ascending graded series of alcohol by a follow-up routine application method. Then, they were embedded in paraffin and prepared for histopathological examination as five micrometer thick serial sections stained with hematoxylin and eosin. Histopathological evaluation was performed under a photomicroscope (Olympus BH 2, Tokyo, Japan).

2.7. Characterization of imipenem/cilastatin – induced lithiasis

The infrared absorption spectra were recorded using Fourier transform infrared spectroscopy (FT-IR) as described by Cuervo et al. (2013).

2.8. Statistical analysis

All data were analysed with the Statistical Package for Social Sciences (PASW Statistics 18). Results were expressed as mean ± standard deviation (SD). Statistically significant difference was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Probability values $p < 0.05$ were considered to be statistically significant.