Evaluation of some biochemical parameters in relation to acute renal failure
Department of Biochemistry, Faculty of Veterinary Medicine, Suez Canal University

Abstract
Glomerular filtration rate (GFR) is the best overall index of renal function in health and disease. Recently, Cystatin C (Cyst C), a low molecular weight protein freely filtered through the glomerulus, and almost completely reabsorbed and catabolized by tubular cells, has been proposed as a new and very sensitive serum marker of change in GFR. This study investigated the role of Cystatin C and other biochemical parameters in the diagnosis of acute renal failure in male rats treated with cisplatin chemotherapy.
The present study was carried out on a total number of 56 adult male Spraque Dawley rats, weighing 150-200g, divided into 2 groups: (1st group as a control group, 2nd group used as acute renal failure model). Cisplatin induced acute nephrotoxicity that revealed by increase serum creatinine, urea, BUN and Cystatin C. It also produce a disturbance in electrolyte through decrease sodium and increase potassium levels. Cisplatin also produce a disturbance in mineral metabolism through hyper phosphatemia and hypocalcemia. So these finding suggested that serum Cystatin C (a cysteine protease inhibitor) has been suggested as a new marker for detection of acute renal failure.

Introduction
The search for new biomarkers for acute renal failure is evolving rapidly with advancement in modern technologies. With better biomarkers we will have the ability to detect acute renal failure earlier, identify subclinical cases, provide prognostic information on the course of renal impairment, identify the nephron segments most affected, guide timing of therapy, assess response to therapy, and screen patients at risk for acute renal failure. During treatment with chemotherapeutic agents such as cisplatin (CDDP), oncologists must monitor kidney function because alteration in renal function may lead to impaired metabolism and accumulation of chemotherapeutic agents and their metabolites (Patterson and Reams, 1992). Because of proximal and, probably, distal tubular cell necrosis, the nephrotoxicity of CDDP is dose-related and cumulative; it depends on the extent of diuresis and preexisting alterations in renal function. Therefore, early recognition of significant renal injury is needed for safe and effective use of this agent (Weiss, 1997).
Glomerular filtration rate (GFR) is the best overall index of renal function in health and disease. Recently, Cystatin C has been proposed as a new and very sensitive serum marker of change in GFR (Rose, 1984)

Cystatin C is a nonglycosylated 13-kDa basic protein of the Cystatin superfamily of cysteine proteinase inhibitors with widespread distribution in biological fluids. It consists of 120 amino acids and is produced at a constant rate by all nucleated body cells (Abrahamson et al., 1990 and Grubb et al., 1992). This protein is not significantly influenced by inflammation unlike creatinine, it is not affected by muscle mass and does not face the same problems with analytical interference as creatinine (Khyse-Anderson et al., 1994).

In the present study, we measured serum concentrations of creatinine and Cystatin C and other biochemical parameters in acute renal failure and we also investigated the potential of Cystatin C as a screening indicator for decrease renal function in in rats treated with cisplatin chemotherapy.

**Material and methods**

**Experimental Animals:**
A total number of 56 adult male Spraque Dawley rats, weighing 150-200g were used in this study. The animals were obtained from the Laboratory Animals Research House at college of Veterinary Medicine, Zagazig University.

**Diet & Management of rats:**
Rats were kept for 4 weeks for acclimatization, and they were housed in separate metal cages under controlled environmental and nutritional conditions (20°C and 55-60% relative humidity). Throughout the period of experiment they were maintained on a standard diet contains corn starch 668, casein 180, DL-methionine 2, corn oil 50, salt mix 40, vitamin mix 10 and cellulose 50 g/kg diet, which approximately equals to 59% carbohydrate, 3% fat, 17% proteins and 21% water-mineral-cellulose (Tae–Yoal et al., 1995 and Brichard et al., 1996). The animals had free access to water and food.

**Drug and chemicals:**
Cisplatin (cis-diamminedichloroplatinum)®:
Cisplatin (cis-diamminedichloroplatinum) was obtained from Merk sharp & Dohm Co. for Pharm. & Chem. Ind. USA. It is clear, colorless solution for intramuscular injection. Each 1ml of solution contains 1mg of cis-diamminedichloroplatinum.

**Experimental Design:**
Rats were randomly divided into 2 main groups "a&b groups" each group consist of 28 rats placed in individual cages and classified as follows:
Group (a) (Control group): (n=28) rats, injected once I. P with saline 6ml/kg b.w
Group (b) (Acute renal failure model): (n=28) rats were injected once I.P with Cisplatin 6ml/kg b.w (Bagnis et al., 2001).

**Sampling:**
Blood samples were collected at 1st, 2nd,3rd and 4th days (7 rats from acute group & 7 rats from control group each day) after overnight fasting
from the medial canthus of eye using micro-hematocrite tubes. The blood samples was collected into a clean and dry screw capped centrifuge tubes and left to clot at room temperature, then centrifugated at 3000 r.p.m for 15 minutes to separate clear serum samples for determination of the different biochemical parameters.

Methods

Determination of serum creatinine:
Serum creatinine was determined by colorimetric Jaffe Kinetic technique according to the method described by Butler (1975) and vasiliades (1996).

Determination of serum urea and BUN:
Serum urea was determined by Enzymatic–UV Kinetic technique according to the method described by Fawcett and Scott (1960).

Determination of serum calcium:
Serum calcium was determined by direct colorimetric complexometric test (Arsenazo III) according to the method described by Bauer (1981).

Determination of serum phosphorus:
The determination of serum phosphorous was determined by phosphomolybdate U.V end point according to the method described by Daly, (1972).

Determination of sodium (Na⁺) and potassium (K⁺):
Sodium (Na⁺) and potassium (K⁺) analysis were accomplished by emission flame photometry after suitable dilution as described by Dean (1960).

Determination of IGF1 hormone:
The concentration of IGF1 in serum was measured using specific enzyme-linked immunosorbent assay (ELISA) kit (Biosource Europe S, A., Nivelles,Belgium). The measurement carried out according to the manufacturer's instruction (Blum et al.,1993).

Determination of serum Cystatin C:
The determination of serum Cystatin C was determined by Enzyme Immunoassay Kit according to the method described by Pergande and Jung,(1993)

Determination of serum Parathyroid hormone:
Serum Parathyroid hormone was determined by the electro-chemiluminescence immunoassay according to the method described by Berson et al (1963).

Statistical Analysis:
All data were subjected to statistical analysis according to Snedecor and cochrane (1982) by using a computer program (Costate) on way compleley randomized. Analysis of variance test "F Test" (ANOVA) treatment means where then compared by the least significance test "LSD" at 0.05, 0.01 and 0.001 level of probability.

Results

Serum urea, BUN, Sodium (Na⁺) and IGF1:
Cisplatin induced harmful effect on the kidney as evidence by a significant (P<0.01) increases in the concentrations of serum urea and BUN at the 3rd day and highly significant (p<0.001) increase at the 4th day when compared with saline-treated controls, also significant (p<0.01) decrease in serum sodium at 3rd &4th days when
compared with the control group. While Serum IGF1 exhibit no significant difference in Cisplatin treated rats as compared with the control group as shown in figure (1).

**Serum Parathyroid hormone (PTH), Calcium, Phosphorus and Potassium (k⁺):**

Significant (P<0.01) increases in the concentrations of serum PTH and phosphorus while calcium showed a significant (p<0.05) decrease at the 3rd day. Also at the 4th day the result showed highly significant (P<0.001) increases in the concentrations of serum PTH and phosphorus with significant (p<0.005) decrease in calcium when compared with saline treated controls, and serum potassium level showed a significant increase at 3rd & 4th days when compared with the control group as shown in figure (2).

**Serum creatinine and Cystatin C**

Creatinine and Cystatin C showed a significant (P<0.001) increase at 3rd & 4th days when compared with saline-treated controls shown in figure (3).

**Fig (1)** Effects of induction of acute renal failure on Serum Urea and BUN (mg/dL), Sodium (mmol/L) and IGF1 (ng/mL) of rats.

**Fig (2)** Effects of the induction acute renal failure on Serum PTH (Pg/ml), Phosphorus (mg/dL), Calcium (mg/dL) and Potassium (mmol/L) of rats.

**Fig (3)** Effects of induction of acute renal failure on Serum Creatinine (mg/dL) and Cystatin C (ng/mL) of rats.

**Discussion**

Renal dysfunction after Cisplatin treatment is common, and acute renal failure may develop after exposure to a single dose. *(Meyer and Madias, 1994).*

The nephrotoxic effect of cisplatin is probably related to its preferential uptake by the proximal tubular cells of the inner cortex and outer medulla, Other segments of the renal tubule also accumulate cisplatin, but to a smaller extent. Cisplatin concentration in the proximal tubular epithelial cells is five
times higher than the corresponding extracellular concentration (Safirstein et al., 1984).
CDDP is able to generate reactive oxygen species (ROS) such as superoxide anion and hydroxyl radical (Masuda et al, 1994; Baliga et al, 1998 and Matsushima et al, 1998) and to inhibit the activity of antioxidant enzymes in renal tissue (Appenroth et al, 1997). These observations support the hypothesis that the mechanism of nephrotoxicity is related to depletion of antioxidant defense system. Thus kidney decrease in ability to scavenge toxic hydrogen peroxide and lipid peroxide producing during metabolic process (Sharma, 1985)
Cisplatin at dose (6mg/kg b.w once I.P) produces a significant acute nephrotoxicity as evidenced by increases in the concentrations of serum urea and BUN at the 3rd day and highly significant increase at the 4th day when compared with saline-treated controls. The results are in accordance with those of other workers (Bagnis et al, 2001 and Huseyin et al, 2009). These results are due to high doses of CDDP produce impairment of kidney function, which is recognized as the main side-effect and the most important dose-limiting factor (Meyer and Madias, 1994). Cisplatin produces a significant increases in the concentrations of serum PTH and phosphorous while calcium showed a significant decrease at the 3rd day. Also at the 4th day the results showed highly significant increases in the concentrations of serum PTH and phosphorous with significant decrease in calcium when compared with saline-treated controls These result come in agreement with the finding of (Palmer, 2000; Brandi, 2008; Raebel et al, 2010 and Johnson et al, 2010). These results supported the hypothesis that in ARF, hyperphosphatemia may cause hypocalcemia as a result of calcium-phosphate precipitation, resulted in a progressive increase in PTH and a decrease in the calcemic response to PTH (Nancy et al, 2008). Hyperparathyroidism is present even in early renal failure and rats with moderate and advanced renal failure had a decreased calcemic response to PTH and less urinary excretion of phosphorus than normal rats. Thus, it is likely that phosphorus retention was in part responsible for the decreased calcemic response to PTH in rats with renal failure (WILSON et al, 1985 and Purrs et al, 1988).
The acute nephrotoxicity induced by Cisplatin associated with significant decrease in serum sodium & significant increase in serum potassium level at 3rd &4th days when compared with the control group These results come in agreement with the finding of (Palmer, 2000; Lin et al, 2009; Raebel et al, 2010 and Johnson et al, 2010). This can be attributed to the renal insufficiency and tubular dysfunction .The possible involvement of peroxidative damage caused by a reactive oxygen species (ROS) has been suggested in the pathogenesis of CDDP –induced renal failure (Matsushima et al, 1998). In particular, the hydroxyl radical is highly reactive among oxygen radical.
Once excessive hydroxyl radical are released, lipid peroxidation, which cause changes in the fluidity and permeability of membranes, is induced (Schmidy, 1990).

Our results showed that the injection of Cisplatin exhibit no significant difference on Serum IGF1 in cisplatin treated rats as compared with the control group. These results is due to the serum concentrations of IGF-1 (or soma-tomedin C as it was then called) are usually normal or near normal, the bioactivity of this hormone only is reduced in uraemic sera (Phillips and Kopple, 1981) and gene expression changes were not observed in the liver following cisplatin administration (Qihong et al, 1997).

Serum creatinine and Cystatin C showed a significant increase at 3rd & 4th day when compared with saline-treated controls. These result come in agreement with the finding of Phillips and Kopple (1981). These due to high doses of CDDP produce impairment of kidney function evidenced by increases in the concentrations of serum creatinine and Cystatin C.

Cystatin C has a low molecular weight (approximately 13.3 kilodaltons) and positive charge at physiological pH levels facilitate its glomerular filtration. Subsequently, it is reabsorbed and almost completely catabolized in the proximal renal tubule (Grubb, 1992 and Tenstad et al, 1996). Therefore, because of its constant rate of production, its serum concentration is determined by glomerular filtration (Pergande and Jung, 1993) as kidney function and as glomerular filtration rate decline, the blood levels of Cystatin C rises.

Although creatinine is the most widely used biomarker of kidney function, its levels can vary with muscle mass and protein intake in contrast to Cystatin C. Therefore Serum levels of Cystatin C are a more precise test of kidney function than serum creatinine levels (Dharnidharka et al, 2002 and Roos et al, 2007) as Cystatin C levels are less dependent on age, sex, race and muscle mass as compared with creatinine.

From the obtained results it could be concluded that Cisplatin "chemotherapeutic agent for treatment of solid cancers" induce acute nephrotoxic effect that occured though:
- DNA damage Stephen and Trzaska, (2005) and (Pruefer et al, 2008)
- RNA damage Peter and Maria (1998)
- Increase the amount of free radicals and decrease the antioxidants in the cells Sharma (1985)

Cisplatin induced acute nephrotoxicity which was revealed by increase serum creatinine ,urea and BUN and Cystatin C . It also produce disturbance in electrolyte through decrease sodium and increase potassium. Cisplatin also produce disturbance in mineral metabolism through hyperphosphatemia and hypocalcemia. In recent years, it has been suggested that GFR can be predicted based on the serum Cystatin C concentrations as serum Cystatin C concentration is not influenced by gender, age and muscle mass in contrast to creatinine. This study recommends the use of Serum
Cystatin C as a new marker for the diagnosis of acute renal failure.

Reference


Daly, J.A.(1972): "Etringshausen, G., Direct method for determining inorganic phosphorous in serum with the centrifichem". Clin.chem.18-263.


Ibrahim et al


Pruefer, F.G.; Lizarraga, F.; Maldonado, V. and Melendez-


Ibrahim et al