Sodium selenite attenuated cisplatin-induced toxicity in rats: Role of electrolytes homeostasis

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Sodium selenite (1 mg/kg body weight, ip) for 10 consecutive days treatment showed marked increase in intra-erythrocytes K⁺ and plasma Na⁺ level while slight increase in Na⁺ K⁺ ATPase level. No mortality was observed at this dose of sodium selenite. However, sodium selenite pretreatment partially restored the Na⁺ K⁺ ATPase and intra-erythrocytes and plasma sodium level, while completely restored the intra-erythrocytes K⁺ and plasma Mg²⁺ level. No change was observed in plasma Ca²⁺ level. Thus sodium selenite successively attenuated the Cisplatin-induced electrolytes alterations and toxicity by exerting the stress response of sodium.

Keywords: Cisplatin, Electrolytes homeostasis, Na⁺ K⁺ ATPase, Sodium selenite

Cisplatin, cis-diaminedichloroplatinum II (CDDP), is an important chemotherapeutic agent commonly used as radiosensitizer for cervical cancers¹ and as a component of consolidation therapy for many solid tumors². Despite the dose dependency of the Cisplatin antineoplastic effect, the risk for nephrotoxicity often precludes the use of higher doses to maximize therapeutic effect. Renal dysfunction after Cisplatin treatment is common, and acute renal failure may develop after exposure to a single dose. Chronic conditions are characterized by polyuria and renal electrolyte wasting, especially persistent hypomagnesemia³. Multiple types of membrane transporters contribute to chemo sensitivity and resistance of tumor cells. Cisplatin, a water soluble drug, cannot cross the plasma membrane unless they piggy back on to the membrane transporters, or enter through hydrophilic channels in the membrane⁴; the resistance may result from decreased activity of the uptake transporters or alternatively enhanced efflux. Cisplatin influences the cell processes by interfering with cytoplasm enzyme functions, modifying permeability, ion transport and ligand receptor interactions at the cell membrane surface. Intracellular uptake of Cisplatin by organic ion transporters plays key role in Cisplatin mediated nephrotoxicity include inhibition of DNA synthesis, oxidative stress and perturbation of calcium⁵.

Antioxidants, as adjuvant in chemotherapy, have ability to induce programmed cell death—apoptosis⁶. Selenium in the form of selenocysteine or selenomethionine, functions as an essential micronutrient and is known to suppress carcinogenesis⁷. Keeping this information in view, the present study has been undertaken to examine the effects of Cisplatin on membrane electrolytes and enzymes, to relate these effects to cellular toxicity and to evaluate the ameliorative effects by administration of antioxidant sodium selenite as potential protective agent against the Cisplatin induced toxicity in experimental rats model.

Animals—Male Albino Wistar rats (200–250 g body weight), purchased from the animal house of International Center for Chemical Biological Sciences, Karachi, Pakistan were used. Animals were acclimatized to the laboratory conditions for one week before the start of experiment and caged in a quite temperature controlled room (23°C±4°C). Rats had free access to water and standard rat diet. The experiments were conducted in accordance with ethical guidelines of internationally accepted principles for laboratory use and care in animal research (Health Research Extension Act of 1985)⁸.

The animals were divided into following 4 experimental groups of 6 rats each:

Group I: (untreated control) Control group, received distilled water for 10 days

Group II: nephrotoxic group (+ve Cisplatin control) received Cisplatin (3 mg/kg body weight, ip) for 5 alternate days

Group III: treated with sodium selenite (1 mg/kg body weight, ip) for 5 alternate days

Group IV: treated with Cisplatin (3 mg/kg body weight, ip) plus sodium selenite (1 mg/kg body weight, ip) 10 min before Cisplatin administration for 5 alternate days

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Plasma separation—After 48 h of last dose of Cisplatin and sodium selenite, animals were sacrificed by decapitation. Blood was collected in lithium heparinized tube and plasma separated by centrifuge at 2000 rpm for 5 min. Plasma was stored at −70°C till analysis of electrolytes.

Erythrocyte membrane preparation—Buffy coat from plasma was aspirated and discarded. Erythrocytes were washed thrice at room temperature by suspension in the MgCl₂ solution (112 M) and centrifuged at 450 g at 4°C for 5 min. Final supernatant was retained for the estimation of intra erythrocyte sodium and potassium concentration. Neither of the electrolytes was detectable in the final wash. The packed red cells extracted by centrifugation at 4°C, 450 g for 15 min were resuspended and diluted in 25 volumes of 0.011 M Tris-HCl buffer, pH 7.4. The hemolyzed cells were then centrifuged for 30 min at 12,000 rpm at 4°C and the membrane pellet was resuspended in 30 mL of 0.011 M Tris-HCl buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was ~4 mg protein/mL of Tris buffer. The concentration of protein was estimated by Biuret Method. The membrane suspension was stored at −80°C until the assay was performed.

Analytical method

Plasma urea and plasma creatinine and intra-erythrocytes sodium and potassium were estimated. Erythrocyte Na⁺-K⁺-ATPase activity was measured as per Denis et al. Plasma electrolytes (sodium, potassium, calcium and magnesium were estimated following Hallry and Sky Peck.

Results are presented as mean±SD. Statistical significance and differences from control and test values were evaluated by Student’s t-test. Statistical probability of P<0.001, <0.01 and <0.05 were considered to be significant.

The results showed decreased magnesium, calcium and sodium content in plasma (Fig.1 d,e,f), increased potassium content in plasma (Fig. 1g) and decreased content in red blood cells in Cisplatin treated rats (Fig. 1b) suggesting electrolyte disturbance with cisplatin toxicity. Electrolyte disturbances, particularly hypomagnesaemia and hypocalcaemia, may occur as a result of renal toxicity after Cisplatin administration reported in rats. Cisplatin causes direct injury to mechanisms of magnesium reabsorption in the ascending limb of the loop of Henle as well as the distal tubule, consequently increases the urinary loss of magnesium. Magnesium deficiency, however means impaired effectiveness of the Na⁺-K⁺ pump and ATPase (Fig. 1c), magnesium impairment followed by potassium and finally insufficient potassium can be pumped into the cell. Therefore, the potassium gradient cannot be maintained. Potassium leaves the cell and to compensate it sodium and hydrogen enter into the cell. The observed decrease level of plasma magnesium followed by potassium after the Cisplatin administration thus may play a role in electrolytes disturbances.

Fig. 1—Intra-erythrocyte sodium (a) potassium (b) Na⁺-K⁺-ATPase (c) plasma calcium (d), magnesium (e), sodium (f), potassium (g) levels in rats [P values: *,+,<0.01; **, ++ < 0.05; ***, +++ <0.001; * compared with control; + compared with cisplatin control]
It is possible that observed Cisplatin induced decrease in plasma magnesium may decrease Na⁺-K⁺-ATPase activity (Fig. 1c). Magnesium act as a substrate for ATPase and helps to stabilized in forming the ATP-Mg complex. Similarly, a marked decrease in Ca²⁺ level was recorded (Fig. 1c). Ca²⁺ plays a key role in regulating cell metabolic processes by stimulating or inhibiting key enzymes and maintaining Na⁺-K⁺-ATPase in an active state. The increased level of intracellular Ca²⁺ shuts down the Na⁺-K⁺-ATPase, an integral glycoprotein of plasma membrane. Guarino et al. implicated this inactivation of ATPase as responsible for kidney toxicity. Cisplatin stimulates the state 4 respiration, uncoupling of oxidative phosphorylation and inhibit ATP synthesis. In the present study Cisplatin disturbed the electrolytes homeostasis, implicating the activity of Na⁺-K⁺-ATPase.

Epidemiological studies, experimental research and clinical trials have demonstrated that selenium has anti-carcinogenic activities. The present study shows protective effect of sodium selenite on disturbed electrolytes when compared with Cisplatin control. Sodium selenite pretreatment restored the sodium content of red cells (Fig. 1a).

Stress response by sodium salt loading caused hydration and protected the Cisplatin induced nephrotoxicity. The mechanism by which salt exerts its protective effect is unknown; however, several theories have been proposed to explain the protective effect of hydration against Cisplatin nephrotoxicity. Vermulen et al. have suggested that hydration protects the kidney by increasing the rate of Cisplatin excretion. These investigators demonstrated that the sodium, rather than the selenite, is responsible for protection. The salt does not exert its effect by interacting directly with Cisplatin, but rather by altering the osmolality of extracellular solutions, modulating a stress response within the cell that alters the sensitivity of renal cells to Cisplatin toxicity. Extracellular changes in the osmolality triggered cell swelling or cell shrinking. Changes in the osmolality have been shown to alter the chromatin structure and free radical induced DNA-protein crosslinks. Therapeutic effect of Cisplatin is attributed to the formation of adduct with DNA. Alteration of chromatin structure by the osmolality may alter the accessibility of platinum to DNA.

The nephrotoxicity is due to the formation of conjugate to glutathione, form reactive thiol which is toxic to the cell. Metabolism of glutathione conjugate and the cystenyl-glycine conjugate occurs extracellularly, the cysteine conjugate metabolized intracellularly to reactive thiol by cysteine-S conjugate-lyase. The osmotic stress response could be occurring any of several steps in this pathway, thus help to reverse the toxic effect of Cisplatin.

Shafaq and Tabassum reported that Cisplatin disturbed balance between oxidation and antioxidation mechanism which consequently effects the membrane electrolytes. Present results showed that disturbances occur in plasma and membrane electrolytes in Cisplatin treated rats, possibly reversed by sodium selenite by exerting the stress response of sodium salt.

In conclusion, the results suggest that Na⁺-K⁺-ATPase dysfunction and changes in intra-erythrocytes and plasma electrolytes induced by Cisplatin is implicated toxicity. The antioxidant sodium selenite on Cisplatin-induced toxicity in rats was associated with the reverse in alteration of membrane electrolytes suggesting that selenium ameliorates the adverse side-effect associated with electrolytes disturbance leading to cellular toxicity by Cisplatin.

References