

COMPARATIVE STUDIES ON CISPLATIN AND ETOPOSIDE TREATED LIVER AND KIDNEY PROTEIN EXTRACT OF RAT UNDER SDS -PAGE GEL SEPARATION

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ABSTRACT

Objective : Our previous studies we have documented that cisplatin dose at 0.4 mg/kg i.p for 8 weeks caused alterations at both histopathological and biochemical level however etoposide dose at 1mg/kg/ i.p for 8 weeks did not cause any hepatotoxic or nephrotoxic effect. These studies aims further the changes in protein pattern of liver and kidney tissue after drug treatment which support previous findings of any free radical induced oxidative stress on tissues.

Methods : Sodium dodecyl sulphate and polyacrylamide gel electrophoresis (SDS-PAGE) of liver and kidney protein extract was separated on 5% Stacking gel and 12% Resolving gel and stained by using coomassie brilliant blue R-250.

Results: Hepatic protein pattenen of cisplatin treated rats showed molecular weight 44.46, 13.8, 51.2 kd and other ranging from 16.98-38.9 kd did not show any expression as compared to controls. Inaddition etoposide treatment studies showed molecular weight 14.45-33.11 kd and other ranging from 25.12 to 51.29 kd showed similar pattern of expression compared to controls. Renal protein pattenen of Cisplatin treated rats showed molecular weight 36kd, 29kd, 24kd and 20kd and other in range of 66kd and 45kd did not as compared to controls. Additionally, etoposide studies depicted increase in protein level ranging from molecular weight 66kd, 45kd, 36kd, 29kd, 24kd but 20kd did not increase.

Conclusion: Our studies suggest that these changes in protein levels might have significant role in organ protection.

Keywords: Cisplatin, Hepatotoxicity, Nephrotoxicity, Etoposide, Proteins.

INTRODUCTION

Cisplatin has long been used in clinical trials for its antitumor effects and it is used in treatment of various human malignancies [1]. The antitumor activity of the drug is attributed primarily for its ability to form DNA-adducts [2]. The mechanism of action of the cisplatin includes hydroxylation reactions by drug metabolizing enzymes includes Cytochromes p450. The drug also interacts with glutathione and forms cis-GSH conjugates [3,4,5]. The drug is used in salvage chemotherapy in combination with other anticancer agents such as procainamide hydrochloride to reduce the hepatotoxic effect of the drug [4]. However the nephrotoxic effects of the drug limits its use in medical oncology. Cisplatin produces the nephrotoxic effect by increasing the oxidative stress on the organs and reducing the levels of antioxidant levels in kidney [9,10].

The drug etoposide is a semi-synthetic compound derived from the plant podophyllum peltatum has remarkable application in cancer chemotherapy. It is used for the treatment of testicular, ovarian, Hodgins lymphoma and AID's [6]. The mechanism of action of etoposide increase cytochrome levels whereas, glutathione related enzymes did show increase [7].

Current studies are therefore the extension of previous work on cisplatin and etoposide in hepatic and renal tissue of rat. The studies would demonstrate that the drug at its action produces change in protein levels which is then depicted to be the either hepatotoxic or nephrotoxic action of the drug.

MATERIALS AND METHODS

Animals were obtained from Rajudyog Biotechnology Division, Maharashtra (India) and were approved by Institutional Animal Ethical Committee. All the male albino Wistar rats weighing 220–250 g used for the study. Drugs Cisplatin and Etoposide was procured from Dabur India Ltd. All chemicals used were of analytical grade. Standard molecular weight protein marker was obtained from sigma chemicals, USA.

Rats were maintained under optimal condition and received standard pellets and water ad libitum. Animals were acclimatized for a period of 2 weeks and were then treated. Control and

experimental rats were divided into three groups. Group 1 were injected with 0.4 mg of cisplatin per kg i.p. daily, and Group 2 were injected with 1mg of etoposide per kg i.p daily, for a period of 8 weeks. Control group received 0.5 ml of saline i.p.daily along with the treated set of the rats. At the termination of the treatment, rats were given light ether anesthesia and sacrificed. Liver and Kidney was dissected out, washed in ice-cold saline, blotted and a homogenate was prepared in lysis buffer (50mM Tris-cl,pH .7.5, 150mM Nacl,2mM EDTA, 0.5mM DTT, 1.0mM PMSF) followed by addition of 1% Triton X-100 and 2% NP-40 with final concentration of 5ml/gm tissue with lysis buffer. Samples were sonicated for 6 cycles of 30secs pulse with intermittant cooling for 60secs on ice. After sonication samples were centrifuged at 10,000 rpm for 30mins, at 4 °C. Cell fractions used for determining protein concentration by Folin Lowry (1951). Standard molecular weight protein marker's ranging from 66kd-14.2kd were used.

SDS-PAGE Electrophoresis

5% Stacking gel and 12% Resolving gel were prepared and used for separation of protein in both cisplatin, etoposide treated groups along with control set.

Sample Preparation

Experimental and control liver and kidney samples containing 20-50 µg of protein were used for loading. Samples were treated with equal volume of tracking dye (0.05M Tris-cl, pH 6.8, 2% SDS, 10% glycerol, 0.25% bromophenol blue as a indicator dye).

SDS reducing buffer was prepared by adding 50µl of 2-mercaptoethanol to 1ml of stock solution as 2x tracking dye. Samples were treated for 5 mins, 95°C on heating block followed by quenching on ice and centrifuged at 10,000 rpm, 5 mins. Standard molecular weight protein marker used were obtained from sigma chemicals, USA. The molecular weight of these proteins are as follows : BSA (66kd), ovalbumin (45kd), glyceraldehyde-3-phosphate dehydrogenase (36kd), carbonic anhydrase (29kd), trypsinogen (PMSF treated) (24kd), trypsin inhibitor (20kd), alpha-lactalbumin (14.2kd). All the samples along with standard molecular weight protein were electrophorised.

Electrophoresis was carried out with a constant current of 60 mA till samples were stacked in stacking gel and then to 31mA till bromophenol blue marker reached at the bottom of the gel. The gel was removed by dismantling unit and was subjected for coomassie brilliant blue staining.

Staining and Destaining of gel

The protein in gel was fixed by using Destaining I solution for 5 mins and stained in 0.25% coomassie brilliant blue R-250 in 10% acetic acid and 45% methanol for 2 hrs. Gels were destained by repeated washing in 10% acetic acid and 50% methanol which was used to determine molecular weight of protein. The protein that are expressed were compared among control, cisplatin and etoposide treated groups.

RESULTS AND DISCUSSION

In our previous studies we have demonstrated the mechanism of action of Cisplatin. We have determined that cisplatin at this dose produced increase in lipid peroxidation followed by reduction in glutathione levels in hepatic tissue of rat [5]. Current studies on SDS-PAGE reveals protein expression in cisplatin liver. Our figure 1 depicts protein patterns with molecular weight of 44.46, 13.8, 51.2 kd, 38.9, 32.36, 28.84, 25.12, 16.98 kd were underexpressed after cisplatin treatment as compared to control and etoposide. Thus, it can be said that cisplatin treatment brings major alteration in the expression of some of protein which is indicative of drug effect. This is also in support to morphological alteration followed by change in antioxidant status and increased lipid peroxidation which serves as a indicator of any oxidative stress to tissue [5,9,10,11].

Furthermore, etoposide studies depicted that the drug treatment produced increase in GSH level, without causing any damage to the organ. Our previous studies documents that etoposide did not show any change in lipid peroxidation levels and glutathione status of rat. Moreover, hepatic antioxidant enzymes such as glutathione peroxidase, GGT were unaltered [7]. Studies are further supported by performing SDS-PAGE of etoposide treated liver protein extract. SDS-PAGE studies of etoposide treated liver depicts that drug administered to 1mg/kg to mice did not cause any damage to hepatic tissue [7]. Moreover, SDS-PAGE of etoposide treated tissue showed that protein with molecular wt 66, 33.11, 27.52, 16.2, 14.45 kd (figure 1) are all overexpressed in etoposide treated samples compared to controls and cisplatin. In addition, protein with molecular wt 51.29, 43.65, 38.9, 32.2 and 25.12 kd showed similar type of expression to controls (figure 1).

In kidney cisplatin therapeutic effect caused an increase in cytochrome p450 enzymes [9]. Cisplatin also interacts with GSH to form cis-GSH conjugates which is brought about by an increase in GST followed with non-significant change in lipid peroxidation level [10]. In our histopathological studies we could observe Cisplatin caused acute tubular necrosis and clogging of epithelium [11].

These studies are further supported by studying the protein levels in cisplatin treated renal tissue of rat. In figure 2 the comparative protein pattern for molecular weight of cisplatin treated kidney showed 36kd, 29kd, 24kd and 20kd protein were all downregulated whereas, 66kd and 45kd were upregulated compared to control and etoposide kidney.

Khanam et.al. (2011) reported cisplatin metabolism caused increase in lipid peroxidation, serum creatinine, blood urea nitrogen and alkaline phosphatase levels in rats and alcoholic tinospora cordifolia extract can serve curative action. Other anticancer drug such as doxorubicin is also known to alter GST and GSH content in cardiac tissue of rat indicates that GST serves as oxidative marker in the tissue of rabbits [13]. We have also studied the comparative GST kinetics for both the drugs cisplatin and etoposide in hepatic and renal tissue which showed major alterations in enzyme activities as to control [14].

Furthermore, etoposide is a semi-synthetic podophyllotoxin compound [15,16]. Etoposide treatment at dose of 1mg/kg/day caused changes in antioxidant status however, Cytochrome p450 did not show any change. Histological studies showed dilated proximal convoluted tubules with enlarged lumen and vacuolation in their epithelium around nucleus. In addition to that onset of necrosis and atrophied glomerulus was also observed. Ultrastructural studies showed presence of mitochondria in segments and lumen with broken microvilli. In addition to the morphological changes the biochemical findings depicted significant increase in Glutathione-S-Transferase (GST) and Glutathione peroxidase (Gpx) and decrease in glutathione reductase (GR), gamma glutamyl transpeptidase (GGT) activities however, glutathione (GSH), Catalase (CAT) and Lipid peroxidation (MDA Content) showed non-significant decrease [13], however, protein studies by SDS-PAGE also showed change to that of control protein pattenren. SDS-PAGE shows protein with molecular weight 66kd, 45kd, 36kd, 29kd, 24kd (figure 2) are increased whereas, 20kd protein showed no band when compared with controls.

Thus it can be said that as compared to cisplatin treatment were it had caused major changes in hepatic and renal tissue etoposide drug regimen did not cause significant damage to the organ.

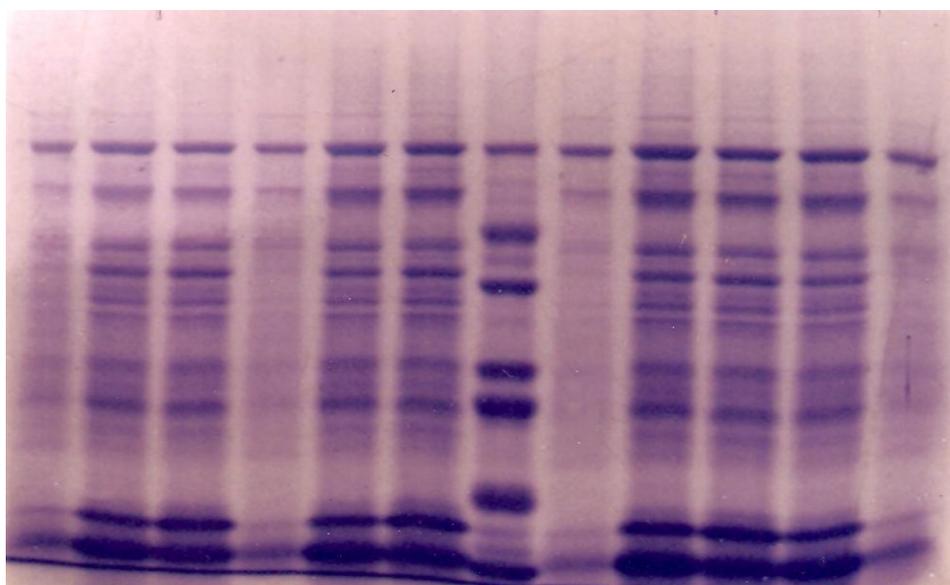


Fig. 1: It shows Coomassie Brilliant Blue staining of SDS-PAGE of Cisplatin and Etoposide treated liver protein extract. Cisplatin liver extract (Lane 1, 4, 8, 12), Control (Lane 2, 5, 9) and Etoposide liver extract (Lane 3, 6, 10, 11). Marker (Lane 7) (66kd, 45kd, 36kd, 29kd, 24kd, 20kd, 14.2kd)

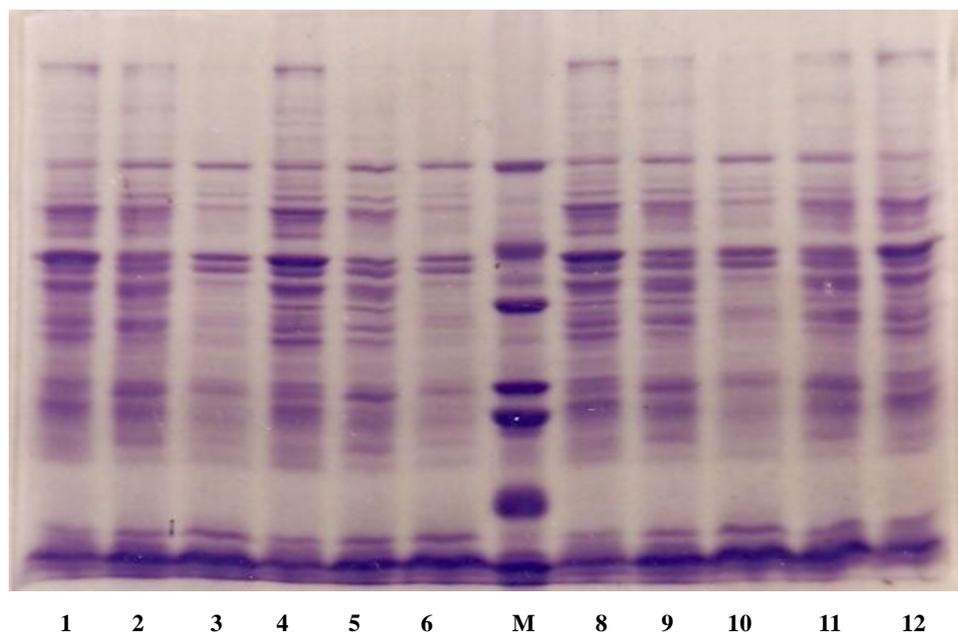


Fig. 2: It shows Comassie Brilliant Blue staining of SDS-PAGE of Cisplatin and Etoposide treated Kidney protein extract. Control (Lane 1, 4, 8, 12), Etoposide Kidney extract (Lane 2, 5, 9, 11), Cisplatin Kidney extract (Lane 3, 6, 10), Marker (Lane 7) (66kd, 45kd, 36kd, 29kd, 24kd, 20kd, 14.2kd)

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