

Original Article



Pharmacokinetics of Cisplatin and its Metabolites Following Intravenous Administration in Cancer Patients of Bangladesh

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Abstract

Background: To determine the mean bioavailability of cisplatin at different time interval after intravenous administration of cisplatin of 18-60 years old 50 male Bangladeshi cancer patients. **Materials & Methods:** Pharmacokinetic and demographic data were collected from 50 various types of male cancer patients received injection cisplatin 50 mg/m² infusion for over 3 hours every alternate week for 3 weeks and mean population bioavailability were determined. **Results:** Statistical analysis from data of HPLC reported that the highest mean plasma concentration of cisplatin was found 428.32 µg/dl (±6.092) after 3 hours and highest mean urinary concentration of cisplatin was found 996.56µg/dl (±1.97) after 12 hours (P<0.05). Highest and lowest concentration of four suspected metabolites (CM2, CM3, CM4 and CM5) were identified in blood and corresponding urine with their specific RT (retention time) and Area (P<0.01) which suggestive of previous work. **Conclusion:** Following I/V administration plasma concentration of cisplatin at different time interval determined the proper dosing dosing interval of the drug and reduce toxicity.

Key words: Bioavailability, Cisplatin, Metabolites and Cancer patients

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Introduction

Cisplatin, cisplatinum, or cis-diamminedichloroplatinum (II) (CDDP) is a platinum-based chemotherapy drug used to treat various types of cancers, including sarcomas, some carcinomas (e.g. small cell lung cancer, and ovarian cancer), lymphomas, and germ cell tumors. React in vivo, binding to and causing crosslinking of DNA which ultimately triggers apoptosis (programmed cell death).^{1,2} Cisplatin is administered intravenously as short-term infusion in physiological saline for treatment of solid malignancies and its dose should be limited by their nephrotoxicity³⁻⁹ and other toxicities such as neurotoxicity, nausea and vomiting, ototoxicity, alopecia, electrolyte disturbance.¹⁰ In pharmacokinetic study, cisplatin is bound to plasma proteins, enters tissues and is slowly excreted in urine with a t_{1/2} about 72 hours. Negligible amount enter brain.¹¹ The cisplatin concentration in proximal tubular epithelial cells is about 5 times more the serum concentration.¹² The disproportionate accumulation of cisplatin

in kidney tissue contributes to cisplatin-induced nephrotoxicity.¹³ Cisplatin is conjugated to glutathione and then metabolized through a glutamyl transpeptidase and a cysteine S-conjugate β-yase-dependent pathways to a reactive thiol, a potent nephrotoxin-β-glutamyl transpeptidase is located on the cell surface, whereas cysteine S-conjugate β-yase is an intracellular enzyme. Cisplatin can form monohydrated complexes by hydrolytic reactions. The monohydrated complex is more toxic to the renal cells than cisplatin but it is not kidney specific. The normal low intracellular chloride concentrations promote its formation.¹⁴

Material and methods

This study was carried out in Indoor patient Department, Department of Oncology, Khwaja Yunus Ali Medical College Hospital, Enayetpur, Sirajgonj, Centre for bioequivalence study, Khwaja Yunus Ali Medical College

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Hospital, Enayetpur, Sirajgonj, Pharmacology Research Lab., Department of Pharmacology, BSMMU, Shahbag, Dhaka From January 2012 to December 2014. A total 50 adult male (age 18-60 years) hospital admitted cancer patients receiving cisplatin were selected for this study. Smoker, alcoholic, female and CVD (cerebrovascular disease) patients were excluded from this study.

Fifty patients were taken for experiment and these patients had been received cisplatin (60-100 mg/m²) as single drug with intravenous constant infusion for 90 min. Blood and urine samples was taken at about five points per patient (at 0,) after 3,6,12 and 24 hours.

Chromatographic conditions: The HPLC-UV diodearray system consisted of Agilent model 1200 series degasser, solvent delivery pump, autosampler, column oven, photo diode array detector. Chromatographic data were collected and analyzed using Chemstation software. A reverse-phase high performance liquid chromatography (HPLC) was used for the determination of cisplatin and its metabolites in plasma and corresponding urine of 40 patients at different time interval (0, 3, 6, 12 and 24 hours). The chromatographic analyses were performed on an Agilent 5 μ m C18 column (150 x 4.6 mm). The mobile phase used for analysis consisted of 20% acetonitril (HPLC grade, E. Mark, Germany) and 80% distilled water and the flow rate was 0.5 ml/min. Separation was achieved at 40°C. The wave length was set at 213 nm (bandwidth 1 nm). Injection of sample (20 μ l) was done using an autosampler. The peak with retention time and areas were defined using software. This is for the quantitative analysis of the drug in ultra filtrate plasma and urine in the presence of nickel chloride as internal standard. Here nickel chloride will be used as internal standard.

Sample collection and preparation of Blood and Urine

Patient blood: A total 250 blood samples of 50 patients was taken for this experiment. Blood samples were taken at about five points per patient. Blood samples were collected at various time points (0, after 1hour, 2 hours, 3 hours, 6 hours, 12 hours, 24 hours and 48 hours) after the end of each infusion. 5 ml x 5= 25 ml blood sample was collected from each patient at different time interval (at 0,3,6,12 & 24 hours). After complete mixing of samples with internal standard, 5 ml of ethyl acetate and vortex mixed for 2 minutes, then centrifuged at 3000 rpm for 15 minutes. 20 μ L aliquots of the supernatant will be directly injected into the chromatography column. Each sample will be analyzed in duplicate. All samples or standard solutions will be stored at -5°C until analyzed.

Patient urine: The urine of a cancer patient was collected after receiving intravenous chemotherapy with cisplatin (60-100mg/m²) drug (0, after 3 hours, 6 hours, 12 hours, 24 hours) respectively. The urine was frozen instantly after collection. For measurement, the urine was diluted in ultrapure water (factor 20).

Determining the amount of cisplatin and their possible metabolites in plasma and urine: Determine the relative peak areas and correct for the molar detection responses of the

individual components. Using the known concentration of the internal standard, calculate the concentration of those components present in the plasma and urine. The structures of the compounds will be elucidated by mass spectrometry.

Isolation and preparation of cisplatin and its metabolites from urine samples:

Separation of cisplatin and its possible metabolites from urine samples of 5 patients and their primary screening for cytotoxicity. Total 20 urine samples of 5 cisplatin treated patients at 3 hr, 6 hr, 12 hr and 24 hr respectively. Each sample consists of 150 ml urine. Evaporation of water was done by rotary evaporator to dryness of the samples. Weight of dried 20 urine samples and preserved for column chromatography.

Column chromatography preparation:

Each sample dissolved in 10 ml methanol and mixed with column grade silica gel and dry in air. The glass column, specially burette (35 x 1.5 cm) was packed with silica gel (Kieselgel 60, mesh 70- 230). When the desired height of adsorbent bed was obtained, a few hundred milliliter of di-chloromethane was run through the column for proper packing of the column. The sample was prepared by adsorbing 2 g of dried urine sample dissolved in 1ml methanol and applied onto silica gel, allowed to dry and subsequently applied on the top of the adsorbent layer. The column was then eluted with di-chloromethane, mixtures of di-chloromethane and chloroform, chloroform-ethyl acetate and ethyl acetate with methanol then methanol with increasing polarity. Primarily 412 samples of 5 ml each were collected from 25 urine samples. After TLC (Thin layer chromatography) analysis, the fractions with similar TLC pattern were recombined and finally seventy three (73) samples were obtained.

Prepared Thin Layer Chromatography of collected fractions:

(Solvent system: chloroform and ethyl acetate in different polarity). PTLC of 73 [Illustration 1] fractions was done. All the column fractions were screened by TLC under UV light and spraying vanillin-sulfuric acid reagent. Mixing the same fraction due to same PTLC character to give rise to 8 fractions (23.9 mg, 27.1 mg, 22.6 mg, 21.3 mg, 19.8 mg, 20.4 mg, 18.7 mg and 17.5 mg) containing probable single compound with some impurities in each fractions. Cisplatin 100 μ g/ml was used as Standard solution. These final 8 fractions were placed in glass chromatography for elucidating single compounds and identified these compounds under UV light and scratch them and dissolved in methanol and collected in small beaker.



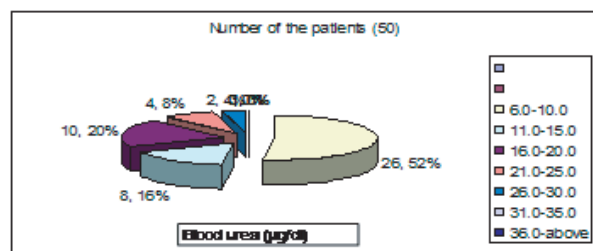
Illustration 1: A few photographs in PTLC of 73 samples

Table I : Rf values of compounds

Compound	Mobilephase	Rf value	Amount	Yield (%)	Compound ID
1	Chloroform :ethyl acetate=80:20	0.812	8.1	4.0	C
2	Chloroform :ethyl acetate=75:25	0.685	7.4	3.7	CM2
3	Chloroform :ethyl acetate=75:25	0.654	8.3	4.1	CM3
4	Chloroform :ethyl acetate=75:25	0.704	6.9	3.4	CM4
5	Chloroform :ethyl acetate=65:35	0.721	8.6	4.3	CM5

Result

Figure 1: Distribution of cancer patient according to the state of uremia



Four percent (n=2), 8% (n=4) and 20% (n=10) of total cancer patients were suffered from severe to moderate to mild hyperuremia respectively indicating strong involvement of renal system of cisplatin toxicity (figure I).

Figure 2: Distribution of cancer patient according to serum creatinine levels

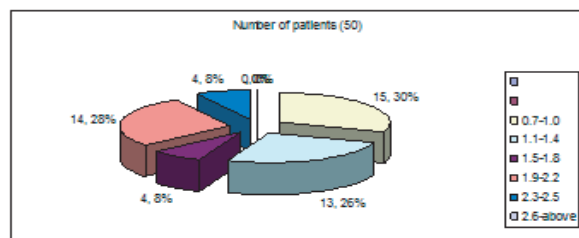


Figure 3 : RT and Area of Cisplatin as standard solution and Nickel chloride as internal standard



Figure 4 : RT and are of cisplatin and its in blood after 3 hour

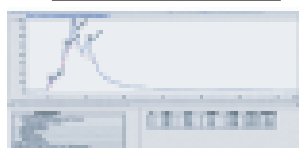


Figure 5 : RT and are of cisplatin and its in blood after 6 hour

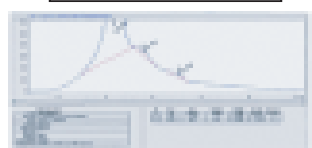


Figure 6 : RT and are of cisplatin and its in blood after 12 hour



Figure 7 : RT and are of cisplatin and its in blood after 24 hour



Figure 8 : RT and are of cisplatin and its in blood after 3 hour



Figure 9 : RT and area of cisplatin and its metabolites in urine after 6 hours



Figure 10 : RT and area of cisplatin and its metabolites in urine after 12 hours



Figure 11 : RT and area of cisplatin and its metabolites in urine after 24 hours

Table II: Cytotoxicity of cisplatin and its suspected metabolites

Name of the compound	LC 50 (µg/ml)
C (Cisplatin, standard solution)	1.8
Cisplatin Metabolite 2 (CM2)	1.2
Cisplatin Metabolite 3 (CM3)	1.08
Cisplatin Metabolite 4 (CM4)	1.18
Cisplatin Metabolite 5 (CM5)	1.25

Discussion

Cisplatin, the most widely used chemotherapeutic agents for the treatment of several human malignancies causing severe nephrotoxicity. Moderate increased of serum creatinine (µg/dl), blood urea (µg/dl) and urea levels were found as 28% (n=14) and 20% (n=10) of the total patients respectively, strongly suggesting that cisplatin or its metabolites produced nephrotoxicities (Table I & II). After continuous infusion of cisplatin (50mg-100mg diluted in 500 normal saline) over 3 hours among 50 patients, statistical analysis from data of HPLC reported that mean plasma concentration of cisplatin after 3 hours was 428.32 µg/dl (±6.092) which was sharply decreased to 3.6 µg/dl (±4.28) after 6 hours possibly due to rapid accumulation of drug in target organs and also unchanged excretion of cisplatin (which proves the previous research work).⁹ Then it was gradually increased followed by decreased to 281.46 µg/dl (±1.88) and 241.98 (±231.96) after 12 and 24 hours respectively (Figure2-5). Corresponding mean urinary concentration was lowest 2.9 µg/dl (±3.15) and 1.68 µg/dl (±1.9) after 3 and 6 hours then rapidly increased to 996.56µg/dl (±1.97) followed by slight decreased to 978.93 µg/dl (±1288.81) after 12 and 24 hours respectively due to excretion of cisplatin. So it could be reported that the highest mean plasma concentration of cisplatin was found 428.32 µg/dl (±6.092) after 3 hours and

highest mean urinary concentration of cisplatin was found 996.56 µg/dl (±1.97) after 12 hours (Figure 6-11). From the evidence of HPLC data sheet, four suspected metabolites (CM2, CM3, CM4 and CM5) were identified with their specific RT (retention time) and Area (Figure 2-5). From HPLC data sheet, the statistical analysis reported that the mean plasma concentration of one suspected metabolite CM2 was gradually increased highest level to 234.64 µg/dl (±6.30) after 12 hours while corresponding mean urinary concentration increased to highest level to 269.43 µg/dl. Another suspected metabolite CM3, the mean plasma concentration after 3 hours was found to 200 µg/dl (±5.65) which was decreasing after 6 and 12 hours to 179.74 µg/dl (±6.43) and 125.35 µg/dl (±4.53) respectively, then slight rise to 207.98 µg/dl (±1.96) after 24 hours, while corresponding mean urinary concentration of CM3 reached maximum level to 1129.04 µg/dl (±9.06) after 3 hours then gradually decreased to 154.03 µg/dl (±5.41) after 24 hours. The third suspected metabolite, CM4 was analyzed. The mean plasma concentration after 3 hours was found to be 154.53 µg/dl (±3.90) which was decreasing to 69.89 µg/dl (±6.54) after 24 hour. The mean urinary concentration of CM4 reached maximum level to 683.08 µg/dl (±8.99) after 12 hours then gradually decreased to 125.77 µg/dl (±1.05) after 24 hours (Figure 2-11).

The mean plasma concentration of last suspected metabolite CM5 was analyzed. The mean plasma concentration of CM5 after 3 hours was found to be minimum 8.02 µg/dl (±8.75) which was sharply increased to 53.86 (±2.73) after 12 hours then gradually decreased to 39.06 µg/dl (±5.44) after 24 hours. On the other hand, the mean urinary concentration of CM5 reached maximum level to 533.38 µg/dl (±0.48) after 3 hours then 513.81 µg/dl (±9.39) after 12 hours and then finally decreased to 153.37 µg/dl (±4.35) after 24 hours (figure 18-19). A marked number of 50 patients were suffered from moderate to severe nephrotoxicities followed cisplatin administration (increased serum creatinine level in 28% of the patients, albuminuria in 16% patients and increased blood urea levels in 20%) which correlate the previous research work.¹¹⁻¹³ Separation of cisplatin and its possible metabolites from urine samples of 5 patients was done by using column chromatography, glass chromatography, PTLC using a range of solvent systems with different polarity and finally their R_f values were done (Table I & Photograph 1).

Conclusion

Present study revealed that the metabolites were found more toxic than cisplatin. So, further study will be designed to identify whether the cisplatin or its metabolites causes nephrotoxicity in cancer patients.

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