

Effect of Treatment Schedule on the Toxicity and Pharmacokinetics of Cisplatin-Doxorubicin Combination in Rabbits

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ABSTRACT

The effects of treatment schedule on the interaction between cisplatin (CDDP) and doxorubicin (DOX) were studied in male New Zealand rabbits. Rabbits were divided into three groups, five animals each. Group I received the two drugs simultaneously while group II animals were treated sequentially with DOX one hour post CDDP administration. A third control group was injected with normal saline. Both drugs were injected as an I.V. bolus of equal doses, 4 mg/kg each, and their concentrations were determined by spectrofluorometry and flameless atomic absorption spectrometry, respectively. Toxicity was assessed biochemically by estimating indices of nephrotoxicity and tissue peroxidative alterations in terms of malondialdehyde "MDA" production levels and non-protein sulfhydryl group contents as well as histopathological examination of kidney, heart and liver tissues. Both treatment schedules showed significant increases in serum creatinine and urea levels ($p < 0.01$). Also, both treatment schedules showed significant increase in MDA production levels and depletion of non-protein sulfhydryl group contents in the kidney, heart and liver tissues ($p < 0.001$). All previous changes were aggravated with sequential administration of CDDP-DOX combination in comparison with the simultaneous one. Furthermore, histopathological examination revealed that sequential CDDP-DOX combination produced significant pathological changes in the rabbit's kidney, heart and liver tissues in comparison with those animals treated simultaneously. These results were confirmed by studying the pharmacokinetics of both drugs. The plasma concentration-time data for both drugs were fitted into an open two-compartment model. DOX pharmacokinetics was significantly altered in the sequentially treated group ($p < 0.001$). The distribution and elimination half-lives for the simultaneously and sequentially treated groups were 0.06 ± 0.0095 h vs 0.104 ± 0.0287 h and 3.48 ± 0.511 h vs 18.96 ± 3.7 h, respectively. The total body clearance in the two groups were 17.64 ± 1.28 ml/min and 3.31 ± 0.68 ml/min, respectively. The mean resident time (MRT) and total area under the curve (AUC) were 4.77 ± 0.689 h and 3.8 ± 0.26 $\mu\text{g/ml} \times \text{h}$ vs 26.82 ± 5.34 h and 20.92 ± 4.76 $\mu\text{g/ml} \times \text{h}$, respectively. The volume of distribution at steady state (V_{ss}) was 5.02 ± 0.58 L vs 5.15 ± 0.291 L, respectively. The pharmacokinetics of

CDDP was not affected by changing the treatment schedule ($p > 0.05$). The only significant change observed was in the distribution half-life, where it appeared to be 0.091 ± 0.054 h and 0.331 ± 0.047 h for simultaneously and sequentially treated animals, respectively. In conclusion, sequential administration of CDDP at one hour prior DOX injection was associated with dramatic changes in the pharmacokinetics of DOX. This might contribute to aggravation of DOX-induced cardiotoxicity as well as hepatorenal toxicity of both drugs.

Key Words: Cisplatin - Doxorubicin - Pharmacokinetic - Lipid peroxidation - Rabbits - Treatment schedule.

INTRODUCTION

Cisplatin (CDDP) and doxorubicin (DOX) are both highly active anticancer agents widely used for the treatment of ovarian cancer, small cell lung cancer and other neoplasms [31]. The clinical uses of CDDP and DOX are limited by their renal toxicity [4,22] and cardiotoxicity [1], respectively. Both drugs are commonly used in combination because of their different organ toxicity and mechanisms of action [11]. The proper sequence of this combination should be started with the administration of CDDP followed by DOX, rather than simultaneous or reversed order of exposure [28]. At the cellular level, the best cell kill is obtained at a one-hour interval between the two drugs, since CDDP improves the penetration of DOX into the core of multi-cellular tumor spheroids (MTS) [20]. Clinically, the combination was reasonably well tolerated and demonstrated notable response rate in patients with advanced or recurrent endometrial carcinoma [6] and in patients with refractory ovarian cancer [12]. Several drugs such as streptozotocin and cimetidine have been

shown to alter the pharmacokinetics of DOX leading to excessive cardiotoxicity [10,13]. Because of the effect of CDDP on the renal function, similar interactions are possible when DOX is administered in combination with CDDP. The interaction between the two drugs in this combination has not been studied in the literature, therefore our work was directed to study the pharmacokinetics and pharmacodynamics of CDDP-DOX in two different schedule combinations.

MATERIAL AND METHODS

A total of 15 male New Zealand White (NZW) rabbits weighing 3-4 kg were used to conduct this study. The study adhered to the National Institutes of Health guidelines for experimental use of animals. The rabbits were divided into three groups: group I received the two drugs simultaneously and group II animals were sequentially injected with DOX one hour post CDDP injection. A third control group of rabbits was injected with normal saline. The marginal ear veins on both sides were cannulated with a catheter (Terumo, 24x3/4-inch, i.d. 0.47x19 mm) and used for administration of CDDP in one ear and DOX in the other. For blood sampling, a third catheter was inserted in the central ear artery opposite to the one used for each drug administration. The animals were prehydrated with normal saline and mannitol (25%) prior to drug administration. The doses of both cisplatin (CDDP) (David Bull Laboratories "DBL") and doxorubicin (DOX) (Farmitalia Carlo Erba, Italy) were 4 mg/kg given as an IV bolus. The samples for CDDP and DOX analysis were collected at 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5 and 24 hours post injection. All samples were collected in heparin coated tubes, immediately centrifuged at 4°C and then directly frozen at (-20°C) until the day of analysis.

Biochemical and histopathological assessment:

Twenty-four hours after DOX administration, a blood sample was collected from each animal and serum separated by centrifugation at 2000 r.p.m for 10 min. Animals were anaesthetized with urethan and heart, kidneys and liver were removed by surgical excision from each animal, washed with cold-saline, blotted with a piece of filter paper, weighed and homogenized (Biohomogenizer) in ice-cold saline. Serum creatinine, urea and albumin levels were measured according to the methods of Bonsnes &

Taussky [9]; Hallt & Cook [18] and Wren & Feichtmeir [33], respectively. Serum calcium was determined using Randox kit (Randox Laboratories Ltd, UK). Lipid peroxidation (Malondialdehyde "MDA") and total non-protein sulfhydryl groups in the organ tissue were determined according to the methods of Okhawa et al. [24] and Ellman [16], respectively. Part from each organ was fixed in 10% neutral formalin, sectioned at 3 µm, stained with hematoxylin and eosin and subjected to histopathological evaluation.

DOX and CDDP measurements:

All plasma samples were thawed just before the assay. The concentration of DOX was measured by quantitative spectrofluorometry (Kontron Spectrofluorometer). Plasma samples (0.1 ml each) were extracted with acid-alcohol solution consisting of 0.45 N hydrochloric acid in 75% ethanol and total drug fluorescence was measured by spectrofluorometry. The clear extracted supernatant was read against a simultaneously run standard curve and the results were determined as (µg/ml) total fluorescence at excitation and emission wave lengths of 470 and 542 nm, respectively [25]. Control plasma from rabbits (Zero time before drug treatment) was treated in the same way as the other samples and read in the spectrofluorometer to correct for any endogenous fluorescence. The sensitivity of the assay was 0.05 µg/ml. The concentration of CDDP was determined by flameless atomic absorption spectrometry using the (Shimadzu^R Japan) system. The assay was sensitive down to a concentration of 30 ng/ml [29].

Data analysis:

Data were analyzed for pharmacokinetic parameters by two-compartment model using the soft "TOPFIT^R" (version number TOPBAS 2.0.0). Several pharmacokinetic parameters were determined such as distribution rate constant (∞), distribution half-life ($t_{1/2 \infty}$), elimination rate constant (β), elimination half-life ($t_{1/2 \beta}$), mean resident time (MRT), volume of distribution at steady state (V_{ss}), zero time concentration (C_0), total area under the curve (AUC) and total body clearance (Cl). The results were expressed as the mean \pm standard deviation of the mean (S.D.). The differences between means of the pharmacokinetic parameters were statistically analyzed by the two-sample student's *t*-test. For the biochemical

study, one-way analysis of variance (ANOVA) was used to test differences between the multiple groups followed by LSD as a post-hoc. The p value of 0.05 or less was taken as the criterion for a statistically significant difference.

RESULTS

Table (1) shows the effect of sequential administration of CDDP one hour prior to or simultaneously with DOX on the renal function of normal rabbits. Twenty-four hours after DOX administration, sequentially treated rabbits revealed aggravation of nephrotoxicity. In comparison with the control untreated rabbits, sequentially treated rabbits exhibited 3.96 fold and 3.66 fold increases, while the simultaneously treated group showed 3.48 fold and 2.91 fold increases in both serum creatinine and urea levels, respectively ($p < 0.01$). The serum creatinine and urea levels of the sequentially treated group were significantly higher than those of the simultaneously treated one ($p < 0.01$). Both treatments had no effect on serum albumin and calcium levels in comparison with the control untreated group ($p > 0.05$).

Table (2) shows the effect of treatment schedule (simultaneous versus sequential) of CDDP-DOX combination on the organ lipid peroxidation (in terms of MDA production levels) and the total non-protein sulfhydryl group contents in the kidney, heart and liver tissues. The percentage change in the total non-protein sulfhydryl group contents and MDA production levels in the organ tissues of both treated groups were significantly different from the control one ($p < 0.001$). In comparison with the control untreated animals, sequentially treated rabbits showed 44% decrease and 368% increase in total non-protein sulfhydryl content and the MDA production in the kidney tissues. However, simultaneously treated rabbits showed 36% decrease and 146% increase, respectively. Sequentially treated rabbits showed 51% decrease and 683% increase versus 32% decrease and 359% increase for simultaneously treated ones in the total non-protein sulfhydryl content and MDA production level in the heart tissue in comparison with those results of the control group, respectively.

Liver tissue of sequentially treated rabbits revealed 53% decrease in non-protein sulfhydryl content and 490% increase in the MDA production level, while simultaneously treated rabbits showed 38% decrease and 30% increase

in the total non-protein sulfhydryl content and MDA production level in comparison with the control untreated ones, respectively.

This was confirmed by histopathological changes observed which revealed that sequential CDDP administration prior to DOX aggravated cardiotoxicity as well as the hepato-renal toxicity of the combination (data not shown).

DOX pharmacokinetics:

The plasma DOX equivalent concentrations after an IV bolus dose of DOX 4 mg/kg were followed up to 24 hours post-injection. The plasma-concentration time curves of both groups were adequately described by two-compartment model (Figs. 1 & 2). Table (3) shows the pharmacokinetic parameters calculated for DOX administered either simultaneously or sequentially one-hour post CDDP. The pharmacokinetics of DOX was dramatically altered in the sequentially treated rabbits (Group II) in comparison with those rabbits that received the two drugs simultaneously. The distribution half-lives ($t_{1/2\alpha}$) and the elimination half-lives ($t_{1/2\beta}$) of the sequentially treated group were 0.104 ± 0.0287 h and 18.96 ± 3.7 h compared with 0.06 ± 0.0095 h and 3.48 ± 0.511 h in the simultaneously treated one, respectively. The difference between the two groups for both parameters was statistically significant ($p < 0.001$). Also, the mean residence time (MRT) significantly increased from 4.77 ± 0.689 h in the simultaneously treated group to 26.82 ± 5.34 h in the sequentially treated one ($p < 0.001$). The calculated plasma DOX concentrations at zero time (C_0) in sequentially treated rabbits were significantly higher (i.e. 3.646 ± 0.497 $\mu\text{g/ml}$ vs 3.02 ± 0.266 $\mu\text{g/ml}$) than those of simultaneously treated ones ($p < 0.05$). The AUC of the sequentially treated group was about 5.5 folds higher (i.e. 20.92 ± 4.76 $\mu\text{g/mlh}$ vs 3.8 ± 0.26 $\mu\text{g/mlh}$) than that recorded for the simultaneously treated group ($p < 0.001$). The total body clearance (Cl) values of sequentially treated rabbits were significantly lower (i.e. 3.31 ± 0.68 ml/min vs 17.64 ± 1.28 ml/min) than those rabbits treated with simultaneous CDDP-DOX combination ($p < 0.001$). The volume of distribution (V_{ss}) exhibited no significant difference (i.e. 5.15 ± 0.291 vs 5.02 ± 0.58 L) between both treated groups ($p > 0.05$).

CDDP pharmacokinetics:

Fig. (2) shows the total plasma platinum (Pt) concentration-time curves following single IV

bolus of 4 mg/kg CDDP administered simultaneously with, or one-hour prior to, DOX. The total plasma Pt concentration declined biphasically and fit into two-compartment model. Table (4) shows the pharmacokinetic parameters calculated for plasma Pt concentrations of simultaneously and sequentially treated groups. The differences between both groups were only

statistically significant ($p < 0.05$) for the distribution rate constant (∞) and distribution half-lives ($t_{1/2 \infty}$) in favor of the sequentially treated group. The distribution half-life was 0.313 ± 0.047 h versus 0.091 ± 0.054 h in the simultaneously treated group. The remaining pharmacokinetic parameters showed no significant differences between both groups ($p > 0.05$).

Table (1): Effect of treatment schedule of cisplatin-doxorubicin combination on the indices of nephrotoxicity in normal rabbits 24 h after doxorubicin administration.

Parameter	Control	Simultaneous CDDP-DOX	Sequential CDDP-DOX
Serum creatinine, (mg/dl)	0.92±0.141	3.20±0.282*	3.64±0.26*#
Serum urea, (mg/dl)	39.80±3.111	115.80±12.80*	145.600±17.18*#
Serum albumin, (g/dl)	4.116±0.05	4.034±0.081	4.038±0.059
Serum calcium, (mg/dl)	8.144±0.40	8.092±1.056	8.280±0.562

All data represent mean values ± S.D (n=5).

* = Different from control group at $p < 0.01$; # = Different from simultaneously treated group at $p < 0.01$.

Table (2): Effect of treatment schedule of cisplatin-doxorubicin combination on the total non-protein sulfhydryl content and tissue lipid peroxidation (in terms of malondialdehyde "MDA" production level) in the organ tissues of normal rabbits 24 h after doxorubicin administration.

Parameter	Control	Simultaneous CDDP-DOX	Sequential CDDP-DOX
<i>Non-protein sulfhydryl content</i> ($\mu\text{mol/g wet tissue}$):			
Kidney	4.02±0.045	2.58±0.075*	2.26±0.237*#
Heart	4.01±0.125	2.71±0.269*	1.978±0.236*#
Liver	4.48±0.098	2.79±0.167*	2.122±0.252*#
<i>MDA production level (nmol/g wet tissue)</i> :			
Kidney	357.25±13.0	879±34.65*	1673.22±243.31*#
Heart	418.78±14.11	1920.34±416*	3279.34±545.56*#
Liver	264.1±15.4	344.61±13.02*	1557.61±50.9*#

All data represent mean values ± S.D (n=5).

* = Different from control group at $p < 0.01$; # = Different from simultaneously treated group at $p < 0.01$.

Table (3): Pharmacokinetic parameters of DOX obtained in two groups (n=5) of rabbits. Group I received DOX and CDDP simultaneously and Group II received DOX one hour post CDDP injection. The doses of both were 4 mg/kg injected as an IV bolus).

Parameter	Simultaneous CDDP-DOX	Sequential CDDP-DOX	Statistically significant $p < 0.001$
α (1/h)	12.00±2.0	7.23±2.7	Yes
$t_{1/2 \alpha}$ (h)	0.06±0.0095	0.104±0.0287	Yes
β (1/h)	0.202±0.031	0.037±0.0068	Yes
$t_{1/2 \beta}$ (h)	3.48±0.511	18.96±3.7	Yes
MRT (h)	4.77±0.689	26.82±34	Yes
V _{ss} (L)	5.02±0.58	5.15±0.291	Yes
Co ($\mu\text{g/ml}$)	3.02±0.266	3.646±0.497	Yes
AUC ($\mu\text{g/ml} \times \text{h}$)	3.8±0.26	20.92±4.76	Yes
Cl (ml/min)	17.64±1.28	3.31±0.68	Yes

The data represent the mean ± S.D. Units correspond to 1/kg.

Table (4): Pharmacokinetic parameters of CDDP obtained in two groups (n=5) of rabbits following i.v. bolus administration of CDDP 4 mg/kg. CDDP was injected either simultaneously with or sequentially one hour prior to DOX administration.

Parameter	Simultaneous CDDP-DOX	Sequential CDDP-DOX	Statistically significant $p < 0.001$
α (1/h)	9.31±4.33	2.24±0.3	Yes
t 1/2 α (h)	0.091±0.054	0.313±0.047	Yes
β (1/h)	0.0078±0.0012	0.013±0.0095	No
t 1/2 β (h)	89.6±12.7	73.23±38.35	No
MRT (h)	125.33±17.01	94.4±49.6	No
V _{ss} (L)	14.36±4.01	13.0±8.40	No
Co (μ g/ml)	12.13±7.9	7.34±1.5	No
AUC (μ g/ml x h)	37.2±14.55	32.03±9.04	No
Cl (ml/min)	1.96±0.62	2.22±0.75	No

The data represent the mean \pm S.D. Units correspond to 1/kg.

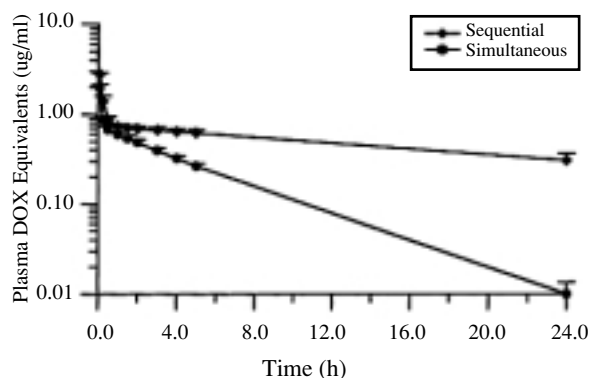


Fig. (1): Plasma DOX levels after IV. bolus administration of DOX 4 mg/kg either simultaneously or sequentially one hour after CDDP administration of 4 mg/kg (Mean \pm SD of 5 experiments).

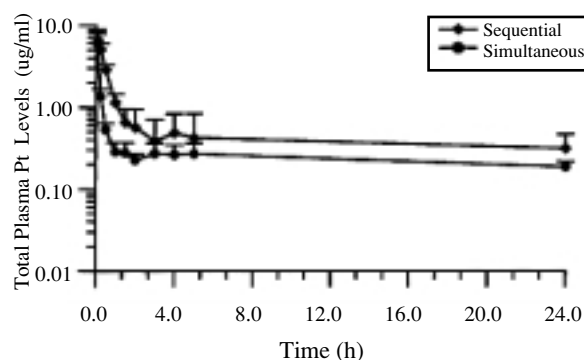


Fig. (2): plasma platinum (Pt) levels after IV. bolus administration of CDDP 4 mg/kg either simultaneously or sequentially one hour after DOX administration of 4 mg/kg (Mean \pm SD of 5 experiments).

DISCUSSION

In the present study, the toxicity and pharmacokinetic changes of DOX-CDDP combination therapy following simultaneous and sequential administration of both drugs were investigated in normal rabbits. The results of the sequentially treated group showed significant increases in serum creatinine and urea levels, as well as tissue lipid peroxidation (in terms of MDA production levels) and decrease in the non-protein sulfhydryl contents of the kidney, heart and liver tissues in comparison with those treated simultaneously as well as the control ones.

At the cellular level, the sequence and time interval between CDDP and DOX appear to be important determinants of antitumor activity. The prior exposure of MTS to CDDP resulted in increased DOX penetration into the MTS core leading to heightened synergism with this sequence [12,28].

Exacerbation of the organ toxicity observed with the administration of CDDP one hour prior to DOX to normal rabbits could be attributed to the fact that CDDP increases catalytic iron both in vivo and in vitro [3] which will enhance semiquinone free radical intermediates [19] generating highly reactive toxic radicals that enhance lipid peroxidation. This was confirmed by histopathological changes observed which revealed that sequential CDDP administration prior to DOX aggravated cardiotoxicity as well as the hepato-renal toxicity of the combination (data not shown). These results were confirmed by evaluating the pharmacokinetics of both drugs in the two treated groups of rabbits. The pharmacokinetic parameters of DOX were significantly altered in the rabbits while received CDDP one hour prior to DOX (group II) compared with those which received the two drugs simultaneously (group I) ($p < 0.001$). It has been reported that intact DOX remained to be the main fluorescent compound in the plasma

as long as 24 hours post-administration [7]. Thus, the concentrations of DOX were measured as total drug fluorescence using quantitative spectrofluorometry. The plasma disappearance profile of DOX, which was followed over 24 h post-injection was consistent with previously reported DOX (HPLC measured) pharmacokinetic investigations in rabbits [2,23].

The pharmacokinetic parameters of DOX, including elimination half-life, MRT and AUC were increased and total body clearance was reduced by more than 5 folds. The effect did not include the volume of distribution at steady state (V_{ss}) where it showed no significant difference between both groups ($p > 0.05$) (Table 3). The volume of distribution had no physiological significance, that it was greater than the total body volume suggests that the drug was segregated and concentrated in concealed compartments [7].

The changes observed in the pharmacokinetics of DOX might suggest interactions in the elimination pathways, since renal and hepatic elimination pathways are involved in the elimination of DOX [8,21]. CDDP-induced renal dysfunction [32] and hepatotoxicity [8,21] are well known phenomena and may be the cause of the changes observed in the pharmacokinetics of DOX. Because of that, drugs such as CDDP, which are known to alter renal and/or hepatic functions, might delay DOX clearance and hence excessive toxicity [10,13]. Literature search did not point to the presence of published studies concerning the pharmacokinetic interaction between CDDP and DOX. However, CDDP has been shown to inhibit the elimination of several drugs such as methotrexate, 67 Ga-citrate and gemcitabine [17,27]. Similar effects on the pharmacokinetics of DOX have been reported when the drug concomitantly administered with streptozotocin and cimetidine [10,13].

The values of both clearance and AUC of the simultaneously treated group are parallel to those reported in the literature for DOX alone [7,23]. It is possible that the pharmacokinetic effect might be behind the toxicity obtained when CDDP is administered one hour prior to DOX. On the other hand, the pharmacokinetic parameters of CDDP were not changed in the presence of DOX. The only parameters affected were the distribution rate constant (∞) and distribution half-life ($1/2\infty$), where the values of

the sequentially treated group were lower and higher than those of the simultaneously treated group, respectively. These differences could be due to difference in protein binding [14,30,34].

In conclusion, sequential administration of CDDP one hour prior to DOX injection was associated with extensive organ toxicity and significant changes in the pharmacokinetic parameters of DOX. The pharmacokinetic changes observed might be a factor in the clinical advantage of sequential administration of this combination. Caution might be considered when the two drugs are administered sequentially as the effect of CDDP on the pharmacokinetics of DOX might contribute to exacerbation of its cardiotoxicity. It might be important to consider the pharmacokinetics of anticancer drugs included in CDDP containing regimens.

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