Changes of essential element content and parrying of cisplatin induced oxidative stress in rat liver by CV247 administration

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Abstract

Metals are accumulated mainly in the kidney during cancer therapy with metal complexes. Since liver is the most important organ for metabolism of the chemicals, the function of it can determine the effectiveness of therapy because of joint side effect of different chemicals in the liver. Therefore our aim was to study the concentration of essential elements Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Zn and antioxidant status in liver of rats after treatment with cisplatin and CV247 product which contains Cu and Mn gluconate, ascorbic acid and Na salicylate. Male Wistar rats (n=40, 175-190 g) were randomly divided into 4 groups (n=10/group). The control group received 1% (w/v) methyl cellulose at 10 mL/kg body weight, p.o. by gastric gavage twice daily for 14 days, while cisplatin was injected i.p. in a single dose of 6.5 mg/kg body weight. The CV247 treated group received 3 mL CV247/kg body weight, p.o. twice daily for 14 days. The forth group of rats was treated with cisplatin and CV247 in the mentioned doses. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used for measuring Ca, Co, Cu, Fe, K, Mg, Mn, Mo, P, S, Zn in the liver and total scavenger capacity and diene conjugate content were also determined. Element depletion was found in the liver of both rat groups treated with CV247, nevertheless the oxidative stress caused by cisplatin was diminished by CV247. But these results pay attention to the importance of essential element, such as Ca and Mg supply as well in cancer therapy.

Keywords: cisplatin; rats; antioxidant effect; essential elements

Abbreviations:
AP-1=transcription factor 1; CDDP=cisplatin, cis-diamminedichloroplatinum II; COX=cyclooxigenase; CV247=copper-manganese gluconate with ascorbic acid and sodium salicylate; DL=detection limit; DV=declared value, F_V=finale volume; GSHPx=gluthatione peroxidase; HHT=12-hydroxy-5,8,10-heptadecatrienoic acid; ICP-OES= inductively coupled plasma optical emission spectrometry; IDL=instrumental detection limit; 6-keto-PGF1α=6-keto-prostaglandin F_1alpha;

LOO•=lipid-peroxid radical; MV=measured value, NF-κB=nuclear factor κB; 15-d-PGJ$_2$=15-deoxy-delta12,14- prostaglandin J$_2$; 19-OH-PGA$_2$=19(R)-hydroxyprostaglandin A$_2$; 19-OH-PGE$_2$=19(R)-hydroxyprostaglandin E$_2$; 19-OH-PGF$_2$ α=19(R)-hydroxyprostaglandin F$_{2alpha}$; PGA$_2$=prostaglandin A$_2$; PGD$_2$=prostaglandin D$_2$; PGE2=prostaglandin E$_2$; PGF2α=prostaglandin F$_{2alpha}$; PGG2=prostaglandin G$_2$; PGH$_2$=prostaglandin H$_2$; PGI$_2$=prostacyclin; R=recovery; RLU=relative light unit; SOD=superoxide dismutase; TXA$_2$=tromboxán A$_2$; TXB$_2$=tromboxán B$_2$; W=weight of sample

Introduction

Platinum complexes such as cisplatin, oxaliplatin and carboplatin are widely used in cancer therapy against several tumors. The most frequently used platinum derivative in head and neck squamous cell carcinoma, testicular, ovarian and bladder carcinomas, and non–small cell lung cancers is cisplatin (cis-diamminedichloroplatinum II, CDDP) [Bose 2002]. Its serious side effect on kidney is well known but it has effect on liver as well, since cisplatin is significantly metabolized by the liver [Liao et al. 2008, Máthé et al. 2014, Naziroglu et al. 2004]. Histopathological investigations justified that cisplatin induces severe liver damage such as degenerative hepatocytes and moderate enlargement of sinusoids [Miyamoto et al. 2007, Lynch et al. 2005]. The prevention of cisplatin side effects was solved with antioxidant treatment with e.g. selenium, vitamin C, ellagic acid, lycopene, resveratrol and macelignan [Bompart 1989, Amaral et al. 2008, Miyamoto et al. 2007, Yüce et al. 2007, Sohn et al. 2008, Sezen et al. 2008]. Vitamin E and L-carnitine separately or in combination could be effective in the prevention of radiation-induced brain and retinal damages [Al-Majed 2007].

The endogenously synthesized L-carnitine effectively inhibits the mitochondrial damage induced by oxidative stress and mitochondria-dependent apoptosis of various types of cells. Carnitine deficiency provokes cisplatin-induced hepatotoxicity in rats [Aleisa et al. 2007]. L-carnitine has also a significant protective effect on the liver and kidney in cisplatin induced oxidative damage by decreasing the lipid peroxidation and elevating the level of glutathione and the activation of glutathione peroxidase, glutathione-S-transferases, and SOD. L-carnitine treatment reduces the sinusoidal dilatation, congestion, and mononuclear cell infiltration of portal area in liver as well as tubular desquamation and dilatation of collective tubules in kidney [Sener et al. 2004].

Toxic effects of metals are displayed via oxidative mechanisms [Stohs and Bagchi 1995], therefore the joint examination of redox and metal homeostasis in the body is necessary. Cisplatin also affects by this mechanism and damages organs, tissues, cells. The most toxic effect appears in the kidney, where Pt is accumulated [Sabolic 2003, Kim et al. 2004] nevertheless in cancer therapy the liver could be damaged seriously because of the side effects and by the several medicines consumed. Therefore investigation of the liver has a great significance in our days because many thousands of medicine can cause liver damage and the joint application of these drugs can cause more serious problems [Meier et al. 2005].

Since several elements (Cu, Zn, Mn, Fe, Se) and metal enzymes (e.g. SOD, catalase, GSHPx) as well as ascorbic acid have a role in the antioxidant system [Zablocka and Janus 2008], we were interested in whether supplementation of Cu and Mn from these metals in organic complex form and ascorbic acid component of the well documented CV247 product can protect the oxidative stress in the liver caused by Pt containing cisplatin and how it is able to modify the metal element content in the liver.

Materials and methods

Test material

Cisplatin (10 mg in 20 mL) was originated from TEVA Pharmaceutical Industries, Petach Tikva, Israel. The CV247 solution (40 mg ascorbic acid, 2 mg manganese gluconate, 2 mg copper gluconate, 35 mg

sodium salicylate per millilitre solution) was obtained from Pharmaserve Ltd, Manchester UK (www.ivymedical.com). Methyl cellulose mucilage (Dow Chemicals) was prepared in distilled water (1%).
Luminol, hydrogen peroxide and microperoxidase were obtained from SIGMA (St.Luis), serum bovine albumin from CALBIOCHEM AG (Lucerne). All other reagents were purchased from Reanal Chemical Company (Budapest).

Animal experiment
Forty 8-week-old Wistar HsdCpb young male rats weighting 175-190 g were randomly divided into 4 groups (n=10/group). The control group received 1% (w/v) methyl cellulose at 10 mL/kg body weight p.o. by gastric gavage twice daily for 14 days. The cisplatin treated group received cisplatin in intraperitoneal injection with a single dose of 6.5 mg/kg body weight in 10 mL/kg 1% methyl cellulose mucin vehicle [Ognjanovic et al. 2012]. The CV247 treated group got CV247 at 3 mL/kg body weight, p.o. twice daily for 14 days. The forth group of rats was treated with cisplatin and CV247 in the above mentioned doses. On the 14^{th} day the rats were weighted then terminally anaesthetised with pentobarbitone.
The animals were kept individually under standard conventional conditions according to European Council Directive 123. The study conformed to the Declaration of Helsinki guidelines and was approved by the local animal ethical committee.
Citrated blood samples were taken from the jugular vein 48 hours before the planned terminal euthanasia after 20 hours starvation. For proving the effect of cisplatin to renal dysfunction, blood urea nitrogen (BUN) and creatinin levels were measured on day 12 besides general routine parameters and essential element concentration in plasma, histological investigation and element content changes in kidney as it was published earlier [Máthé et al. 2013].

Rat samples
The liver was removed, weighed, washed and then homogenized in ice-cold isotonic KCl solution and the protein concentration was set at 10 g/L.

Measurement of protein content of liver homogenates
The protein content of liver homogenates was measured by Lowry et al. The protein concentration of all homogenates was adjusted to 10 mg/ml using bovine albumin as standard for the measurements [Lowry et al. 1951].

Measurement of metal content
Homogenized liver samples were weighed (3.0-3.5 g liver homogenate) into the digestion vessels and digested with 5 mL 65% nitric acid and 2 mL hydrogen peroxide. The digestion was performed in a block digestion system because of the large amount of samples. After digestion and evaporation to small volume, the samples were poured into 10 mL volumetric flasks and were filled up to the mark with bidistilled water. Three liver samples were prepared from each animal.
Inductively coupled plasma optical emission spectrometric (ICP-OES) method was applied for measuring Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Zn and Pt content using a Spectro Genesis ICP-OES (Kleve, Germany) equipment by the method applied earlier [Szentmihályi et al. 2004]. For the standardization of equipment and measurements, Spectro multi-element and Spectrum 3D standards were used. Standards were prepared in the same matrix as the samples. The samples were measured 3 times and blank subtraction was applied.

Metal element concentrations in bovine liver solution (High Purity Standards, CRM BL 411213) were measured for the demonstration of reliability and precision of the measurement (Table 1). The recovery (R) was calculated from the declared (DV) and measured values (MV) as follows: R=MV/DV*100. In the case of Mo and Pt, which had no certified values, the repeatability measurements were performed (five times) with standard solutions of 0.2 μg/mL and 0.5 μg/mL concentrations, and the recovery was calculated from these results (Table 1).
The detection limit (DL) for Pt in the samples was calculated from the instrumental detection limit (IDL) of it, the weights of sample (W) and the final volume (F_V) as follows: DL=IDL*F_V/W. The below detection limit concentration for Pt was marked by the less-than sign (<).

Table 1. Results of element concentration (mean±SD, μg/mL) in reference solution (Bovin liver solution, High Purity Standards, CRM BL 411213) standards and they recovery data

	Declared concentration in reference solution	Standard concentration	Measured (*n*=5)	Recovery (%)
Ca	1.2		1.161±0.119	96.8
Co	0.002		0.002±0.0001	100.0
Cu	2.00		1.990±0.024	99.5
Fe	3.00		2.893±0.018	96.4
K	100.0		92.28±0.21	92.3
Mg	6.00		5.837±0.123	97.3
Mn	0.01		0.0948±0.0004	94.8
Mo		0.2	0.206±0.008	103.1
Na	25		25.09±0.02	100.4
P	110		113.5±1.8	103.2
Pt		0.5	0.493±0.018	98.7
S	80.0		75.50±0.38	94.4
Zn	1.5		1.477±0.028	98.5

Measurement of redox parameters of the liver

Total scavenger capacity (induced chemiluminescence assay) was determined with Berthold's Lumat 9501 luminometer on the basis of a method developed by Blázovics and coworkers [Blázovics et al. 1999]. Chemiluminescent intensity of the liver homogenate was measured and expressed in relative light unit (RLU%) of the standard light (basic chemical reaction in H_2O_2/•OH–microperoxidase-luminol system).
Diene-conjugate content of the liver was determined at 233 nm by spectrophotometry by the AOAC method [1994].
In the liver homogenate malondialdehyde (MDA) levels were also measured spectrophotometrically by the method of Mansour and coworkers [mansour et al. 2006].

Statistical calculations

Means and standard deviations were calculated with MS-Excel, ANOVA and Kruskal-Wallis ANOVA were applied for the determination of significant differences between groups by using STATISTICA 11 software. The level of significance was set at $P<0.05$.

Results

According to the literature cisplatin can generate reactive oxygen and nitrogen species by increasing the activity of the cytochrome P450 system, NADPH oxidases, xanthine oxidase and adenosine deaminase and cisplatin depletes the glutathione and inhibits the activity of antioxidant enzymes [Somani et al. 2000, Jiang and Dang 2008, Sung et al. 2008, Yilmaz et al. 2005, Koc et al. 2005, Ajih et al. 2007]. Nevertheless in our "short term" experiment cisplatin and CV247 alone didn't induce the P450 system in the liver since the ratio of liver weight to body weight didn't change at all or significantly, while the double treatment with cisplatin + CV247 induced it significantly (Table 2).

Table 2. Changes of liver weight/body weight ratio by the administration of cisplatin and CV247

	Control	**Cisplatin**	**CV247**	**Cisplatin + CV247**	**ANOVA sign. at P<0.05**
Liver weight /body weight	0.04214±0.00160	0.04215±0.00281	0.04542±0.02203	0.04775±0.00223	Sign.

Redox parameters in the liver

The total scavenger capacity in the liver of cisplatin-treated rats decreased as the amount of •OH/H_2O_2 radicals in the H_2O_2/•OH-microperoxidase-luminol-system was considerably higher in the liver of rats treated with cisplatin than in the liver of rats in control group (Table 3). The treatment with CV247 did not change notably the total scavenger capacity while the CV247 treatment was able to decrease the formation of free radicals by the effect of cisplatin as the total scavenger capacity was decreased significantly.

The diene conjugate level in the liver doesn't show any significant change while malondialdehyde content in liver increased non-significantly by the effect of cisplatin treatment (Table 3).

Table 3. Redox parameters in the liver

	Control	**Cisplatin**	**CV247**	**Cisplatin + CV247**	**Kruskal-Wallis ANOVA sign. at P<0.05**
Induced chemiluminescence (RLU%)	4.24±2.78	38.22±26.34	3.98±5.13	5.50±10.37	Sign.
Dién conjugate content	0.207±0.036	0.175±0.035	0.203±0.032	0.226±0.036	Not sign.
MDA content (nmol/l)	20.3±5.4	32.9±7.5	25.2±5.6	33.2±8.7	Not sign.

Element changes in the liver

The element content and the significant changes in the liver by the effect of treatments can be seen in Table 4. Significant concentration changes could be seen for the four groups in the case of Ca, Mg, Mn, Mo and Na using ANOVA test at $P<0.05$. The cisplatin treatment increased the Fe concentration in liver, which may cause the increase of free radical reactions as it was shown in Table 3, while CV247 treatment decreased the Fe concentration. By the treatment with CV247 the Ca, Cu, Mg, Mn,

Mo and Sr concentrations in the liver decreased. The joint treatment with cisplatin and CV247 caused significant decreasing in Ca, Cu, Fe, Mg, Mn, Mo, Na concentrations compared to cisplatin group which was worse than in case of the group treated with CV247 alone. This means that the post-treatment with CV247 was not able to protect the liver from the toxic effect of Pt and the combined treatment damaged the liver more seriously. The cisplatin treatment changes several metabolit concentrations, energy homeostases, and therefore ATP concentration also varies (Rodriguez-Enriquez et al. 2009), nevertheless in our experiment only a non-significant decreasing could be seen for P concentration in the liver of group treated with cisplatin+CV247.

Table 4. Element concentration (μg/g) in rat liver measured by ICP-OES

	Control	**Cisplatin**	**CV247**	**Cisplatin + CV247**	**ANOVA sign. at P<0.05**
Ca	6.31±2.36	6.72±1.34	2.36±0.89	1.74±0.53	Sign.
Co	0.005±0.002	0.006±0.003	0.005±0.002	0.004±0.001	Not sign
Cu	0.189±0.047	0.188±0.024	0.168±0.023	0.153±0.029	Not sign
Fe	4.01±1.20	6.55±2.04	3.63±0.34	3.42±0.92	Not sign
K	1275±462	1377±139	1312±336	1261±229	Not sign
Mg	11.23±3.63	11.56±1.46	6.96±0.70	6.19±0.89	Sign.
Mn	0.098±0.028	0.098±0.008	0.080±0.008	0.071±0.012	Sign.
Mo	0.030±0.005	0.028±0.008	0.026±0.003	0.023±0.004	Sign.
Na	20.59±6.24	21.07±3.82	16.39±4.71	15.28±2.89	Sign.
P	129.7±44.7	135.4±19.6	116.4±15.7	109.6±18.8	Not sign
S	71.60±25.21	72.51±12.56	65.43±9.99	62.02±11.91	Not sign
Zn	1.10±0.34	1.09±0.20	1.03±0.18	0.940±0.250	Not sign
Pt	<0.010	0.021±0.001	<0.010	<0.010	

Discussion

The cisplatin treatment elevated the Fe and Pt concentrations in the liver which are unfavorable, since they may induce free radical reactions and elevate the free radical level. The mean part of the increased free radical level may be connected to radicals formed by Fe(II) induced Fenton reaction while smaller part of free radicals comes from the platinum radicals formed by metabolism of cisplatin [Naziroglu et al. 2004, Stohs and Bagchi 1995]. The CV247 product was able to diminish the oxidative stress in the liver which may due to ascorbic acid, salicylic acid, Cu and Mn as well. These metals have significant role in the antioxidant system as a central part of SOD enzymes [Zablocka and Janus 2008] nevertheless a decreasing concentration of Cu and Mn was observed in the liver despite of the supplementation.

Salicylic acid is a good antiinflammatory agent and has direct and indirect antioxidant properties via several mechanisms, e.g. inhibits the prostaglandin (PG) biosynthesis routes [Baltazar et al. 2011, Borges et al. 2013]. Therefore free radicals are formed at the PGG_2 and PGH_2 synthesis steps and inhibits the expression of cyclooxygenases (Figure 1). COX2 inhibition is favorable in the process but the inhibition of COX1 causes unwanted and harmful side effects in the gastrointestinal tract [Agundez et al. 2009, Kneitz et al. 2006, Szentmihályi et al. 2013].

Ascorbic acid content of CV247 also diminishes the free radical formations in different ways. Direct antioxidant activity was proven in several in vitro and in vivo studies, it is able to transform α-tocopheroxyl radical to vitamin E [Packer 1979]. Nowadays its prooxidant activity was also proven via the transcription factors NF kappaB and AP1 activities, which induce gene expression of

antioxidant enzymes and repair mechanisms as it could be seen in Fig.1 [Cooke 2000, 2002, Lunec et al. 2001, Puskás 2000, Blázovics 2007]. The AP1 activation increases the oxidative damage of DNA by oxidation or reduction of thiol group. In this process AP1 binds to DNA which induces the transactivation of DNA repair genes [Kaina 1998]. According to the molecular biological researches in the last years the possible repearing and impering effect of DNA has been known [Cooke 2000, 2002, Lunec 2002]. Ascorbic acid regulates the repair of 8-oxoG by redox way [Kaina 1998, Lunec et al. 2002]. The dose dependent antioxidant and prooxidant properties of ascorbic acid is in connection to the attendant metal ions as well. Ascorbic acid in small concentration has prooxidant activity which is enhanced by the presence of Fe as well [Tsuchiya 1998].

The examination of liver calls attention to that double treatment with cisplatin and CV247 product changes the whole metal homeostasis and the depletion of elements in the liver could be observed. The decreasing concentration of Ca and Mg in both CV247 treated groups and the decreasing concentration of Cu and Mn in cisplatin+CV247 treated group are particularly unfavorable. Furthermore the Ca to Mg concentration ratio in both CV247 treated groups reduced notably from 0.562 (control group) to 0.339 at CV247 treated group and to 0.280 at double treated group. Although this ratio affects against the oxidative stress [Szentmihályi et al. 2013], the decrease of absolute concentration of Ca and Mg is unfavorable in the function of the liver. According to the results it is worthy to think about the supplementation of some essential elements in cancer therapy.

Conclusion: Cisplatin treatment influences the metal element homeostasis and the redox homeostasis as well. The CV247 product acts different modes in different organs. Favorable effects on element and redox homeostasis were observed in plasma and kidney [Máthé et al. 2013]. In the liver it can also diminish the oxidative stress significantly after cisplatin therapy nevertheless the results show unwanted changes of metal element concentration during double treatment as well which can be possible prevented by additional supplementation of metals as Ca, Mg during the treatment.

In this animal experiment salicylic acid was used as an antioxidant component of the agent and it its effectiveness was proved. In human study flavonoids can be applied for avoiding the harmful oxidative effect of cisplatin instead of salicylic acid which supposed to be as good as salicylic acid or better than that of [Blázovics et al. 1992, 2002, 2007]

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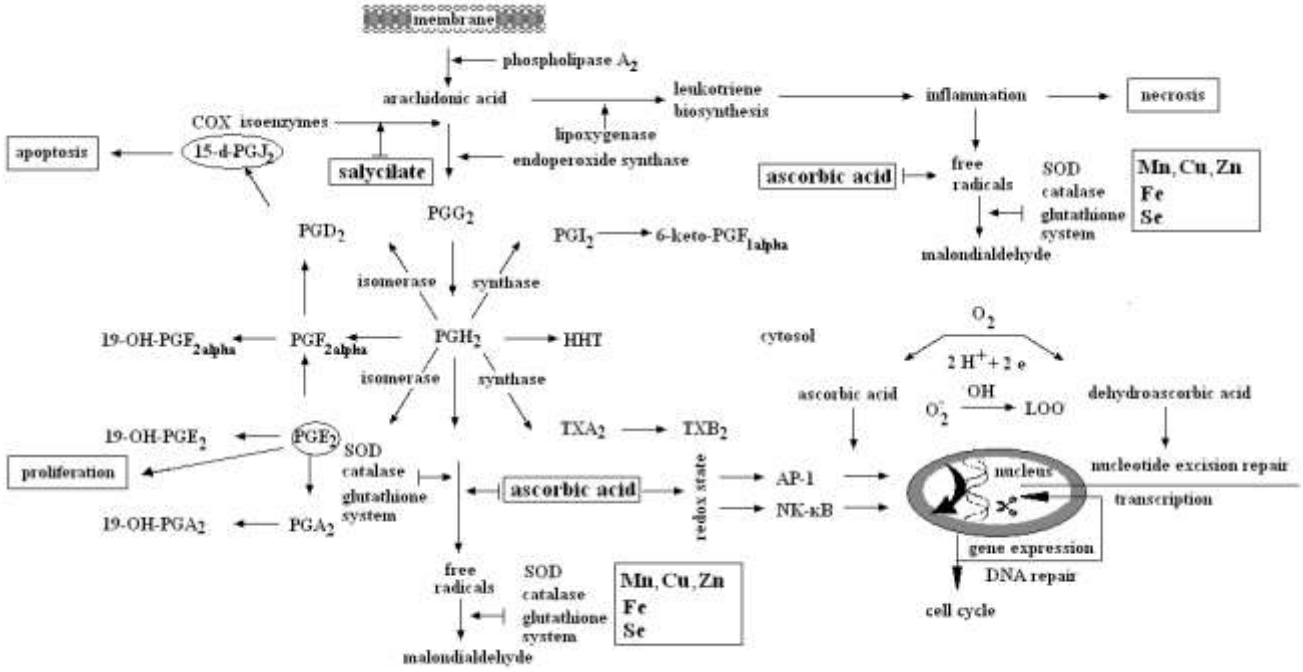

Figure 1. Molecular biological effects of salicylic acid, ascorbic acid and metals of CV247. The figure was designed by the works of Lunec et al. 2002, Blázovics 2007 and Szentmihályi et al. 2013.
(AP-1 = transcription factor 1; HHT=12-hydroxy-5,8,10-heptadecatrienoic acid; 6-keto-PGF1α= 6-keto-prostaglandin F_1alpha; LOO• = lipid-peroxid radical; NF-κB = nuclear factor κB; 15-d-PGJ_2= 15-deoxy-delta12,14- prostaglandin J_2; 19-OH-PGA_2= 19(R)-hydroxyprostaglandin A_2; 19-OH-PGE_2= 19(R)-hydroxyprostaglandin E_2; 19-OH-PGF_2 α = 19(R)-hydroxyprostaglandin F_{2alpha}; PGA_2= prostaglandin A_2; PGD_2= prostaglandin D_2; PGE2= prostaglandin E_2; PGF2α = prostaglandin F_{2alpha}; PGG2= prostaglandin G_2; PGH_2=prostaglandin H_2 ; PGI_2 = prostacyclin; SOD = superoxide dismutase; TXA_2 =tromboxán A_2; TXB_2 =tromboxán B_2)