

## **MODIFICATION OF ENZYMATIC STATUS INDUCED BY XENOBIOTIC ACTION OF CIS-PLATINUM**

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### **Abstract**

*The current study was undertaken to examine the effects of cis-platinum on various enzyme systems in the blood, liver and pancreas, and to relate these effects to different tissues problems. Cis-platinum is known as a most commonly used antineoplastic agent in the treatment of cancer. Data from literature show that cis-platinum administration affects the biochemical homeostasis of organism. We have observed the consequences of accumulation of cis-platinum in blood and also in several different tissues, and we evaluated the modifications which appeared after repeated doses simulating chemotherapeutic treatment in tumor-free rats. For this experiment, we used adults animals from Wistar rats strain divided in four experimental groups and one control group. Wistar rats from the experimental groups were injected intraperitoneally (i.p.) with the drug called Sin-Platinum (i.e. c-DDP), and the animals from control group were injected with saline solution (placebo). Doses have been administrated on days 3<sup>th</sup> and 8<sup>th</sup> of the experiment. On day 13<sup>th</sup> of the experiment the animals were killed. The results showed that the enzymes status is modified proportionally with administrated doses.*

**Keywords:** *xenobiotics, cis-platinum, Wistar rats*

### **Introduction**

Xenobiotics substances are becoming an increasingly problem, because they are new substances, found in organism but which are not normally produced or expected to be present in it (Gârban, 2004).

In oncology are used cytostatics, such as cis-platinum which are considered to be human xenobiotics because human body does not produce them itself (Gârban, 2005).

Cis-platinum, as a compound was the first time described by Peyrone in 1845, and the structure was elucidated in 1895 by Alfred Werner. In 1960s, Rosenberg and he's team research discovered that electrolysis products from a platinum electrode inhibited mitosis in *Escherichia coli* bacteria (Rosenberg, 2000). Cis-platinum is a drug containing a central ion of platinum, which had been used as a chemotherapeutic agent for approximately two decades.

Cis-platinum appears to be an bifunctional alkylating agents that binds to DNA trough covalent bindings and disrupts DNA function. After cisplatin enters the cells, the chloride ligands are replaced by water molecules (Lippert, 1996). Trough this reaction results positively charged platinum complexes that are reacting with the nucleophilic sites on DNA, creating cisplatin-DNA adducts. Cis-platinum action is cell cycle phase-nonspecific, but this alkylating agent also has immunosuppressive, radiosensitizing, and antimicrobial properties. As a consequence of cis-platinum therapy is the disturbance of biochemical homeostasis (Gârban, 2005).

This study was performed to examine the effects of cis-platinum on some blood serum enzymes from Wistar rats.

## **Experimental**

In case of experimental part, we used adults Wistar rats, maintained on pathogen-free conditions, at 22–25°C, at 55–65% relative air humidity, and fed on normal rhythm and standard breeding food and water.

The animals were randomly divided in three groups: one control (C) and four experimental groups (E<sub>1</sub>, E<sub>2</sub>, E<sub>3</sub> and E<sub>4</sub>). Each group contained 8 animals (males and females) with an average body weight (b.w.) of 200 ± 20 g.

Cis-platinum used for this study was the commercially available Sin-Platin (Sindan, Romania). The animals were injected intraperitoneally (i.p.) with 2.0 mg/kg body weight (b.w.) - in case of E<sub>1</sub> group, 4.0 mg/kg b.w., 6.0 mg/kg b.w.- in case of E<sub>3</sub> group and 8.0 mg/kg b.w. - in case of E<sub>4</sub> group. The animals from (C) control group

were injected i.p. with physiological saline solution. Animals Doses have been received the doses on days 3<sup>th</sup> and 8<sup>th</sup> of the experiment, and on day 13<sup>th</sup> were killed.

On the last day of the experiment, after 12 hours of fasting (overnight), and Ketanest anesthesia, the rats were killed, and blood samples were taken for analysis. The samples were taken after laparotomy and puncture of vena cava caudalis. Blood from each animal was collected in a clean centrifuge tubes. The tube was not heparinized before use, and blood was allowed to coagulate and the tube was centrifuged for serum separation. The enzymes, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, alpha – amylase and gama-glutamyl-transferase were assayed trough enzymatic methods, using a Hospitex – Screen Master Plus.

Results are expressed as means  $\pm$  SD and to calculate the statistical significance the *t* test was used as appropriate and *p* values of less than 0,05 and 0,01 were considered as significant.

## **Results and Discussions**

Cis-platinum administration can disturb biochemical homeostasis, and as a consequence, the concentration of blood serum enzymes (Velciov, 2005).

Alanine-aminotransferase (ALT) is a non-tissue specific soluble enzymes, derived from liver and kidney, which catalyses the transfer of amino groups during the transition of amino-acids to alpha-cetoacids. The serum activity is increased during hepatic disease.

During the metabolism of amino-acids aspartat aminotransferase (AST) catalyses the transfer of amino-group. The activity of AST is significantly increased during heart, liver, kidney and muscle diseases (tissue injuries, functional disorders).

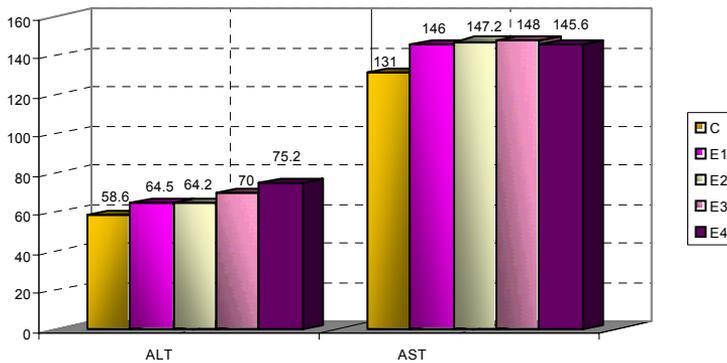
Administration of cisplatin causes changes in some blood serum enzymes status, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in Wistar rats (Chabner, 2001). Mean concentration of ALT and AST from serum blood of Wistar rats (U/L) are presented in Table 1. From the data we have obtained, it can be observed that after cis-platinum administration, both values, ALT and AST are increased in experimental groups compared with control group (Figure 1).

**Table 1.** Concentration of ALT and AST in blood of Wistar rats after cis-platinum administration

Specificare	n	ALT U/L	AST U/L
		$\bar{X} \pm SD$	$\bar{X} \pm SD$
Grup C	8	58.6 ± 6.87	131 ± 19.87
Grup E <sub>1</sub>	8	64.5 ± 5.27	146 ± 4.76
$\Delta \bar{X}$		+5.9	+15
Grup E <sub>2</sub>	8	64.2 ± 12.10	147.2 ± 24.50
$\Delta \bar{X}$		+5.6	+16.2
Grup E <sub>3</sub>	8	70 ± 21.58	148 ± 31.71
$\Delta \bar{X}$		+11.4	+17
Grup E <sub>4</sub>	8	75.2 ± 0.70*	145.6 ± 0.70
$\Delta \bar{X}$		+16.6	+14.6

n – number of animals per each working group; \*p< 0.05 compared with control group

The ALT concentration in experimental group E<sub>4</sub> is significantly higher compared to control group (p< 0.05).



**Fig. 1.** Concentration of ALT and AST in blood of Wistar rats after cis-platinum administration

Distributed in almost every tissue of the body, serum alkaline phosphatase (ALP) levels are of interest in the diagnosis of hepatobiliary disorders and bone disease. Normal alkaline phosphatase levels are age dependent and are elevated during periods of active bone growth.

Alfa – amylase ( $\alpha$ -amilase) is an enzyme found primarily in the pancreas and salivary glands, and released in the digestive tract. Alpha -amylase determinatios are useful in the diagnosis of diseases of pancreas and parotids.

Gamma-glutamyltransferase (GGT) hydrolyses gamma-glutamylated peptides, such as glutatione, and transports aminoacids into the cells. The enzyme concentration is higher in some tumours, especially those with a higher degree of malignancy and resistance to cytostatics.

The variation of concentration of alpha – amylase, gama-glutamyltransferase and alkalyne phosphatase (U/L)in blood serum is presented in table 2.

**Table 2.** Concentration of  $\alpha$ -amilase, gamma-glutamyltransferase, and alkaline phosphatase in blood of Wistar rats after cis-platinum administration

Specification	n	$\alpha$ -amilase U/L	GGT U/L	ALP U/L
		$\bar{X} \pm SD$	$\bar{X} \pm SD$	$\bar{X} \pm SD$
Group C	8	146.20±42.35	7.68±0.27	232.2±11.86
Group E <sub>1</sub>	8	151.50±54.50**	8.95±0.70	224.5±3.74
$\Delta\bar{X}$		+5.3	-1.27	-7.7
Group E <sub>2</sub>	8	256.20±18.07**	9.16±0.41*	219.4±6.51
$\Delta\bar{X}$		+110	-1.48	-12.8
Group E <sub>3</sub>	8	263.40±32.87**	9.26±0.28**	216.4±5.72*
$\Delta\bar{X}$		+117.2	-1.58	-15.8
Group E <sub>4</sub>	8	275.20±7.07**	9.58±1.62**	209.2±4.94**
$\Delta\bar{X}$		+129	-1.9	-23

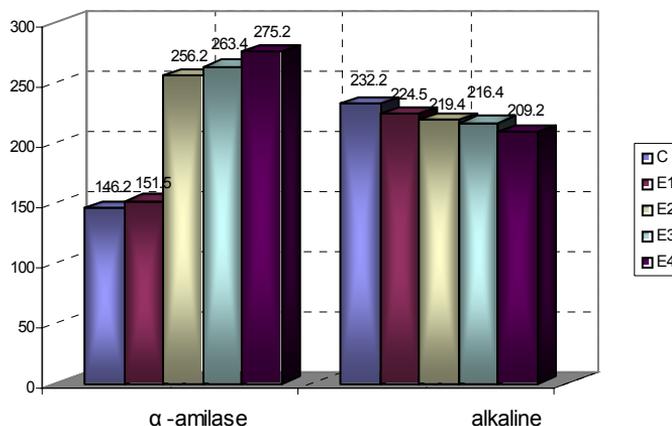
n – number of animals per each working group; \* p < 0.05 compared with control group; \*\* p < 0.01 compared with control group

In case of alkaline phosphatase, serum activity is increased (p < 0.05 in experimental groups in comparison with control groups. This

data can be correlated with elevated values of hepatic enzymes (ALT and AST).

Also, it can be observed an increased serum amylase, in experimental groups in comparison with control group, which can be due to pancreatitis that appeared after cis-platinum administration (figure 2).

The data revealed in Table 2 suggest that GGT is not only increased in experimental groups compared with control group, but significantly higher in experimental group E<sub>2</sub> ( $p < 0.05$ ), and in groups E<sub>3</sub> and E<sub>4</sub> compared to control group ( $p < 0.01$ ).

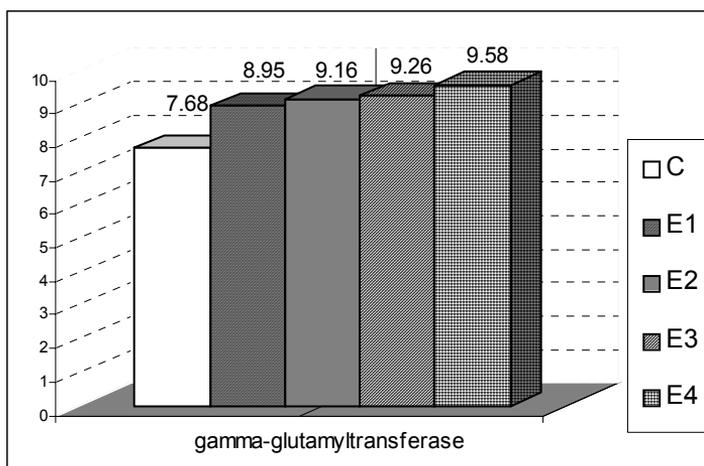


**Fig. 2.** Concentration of  $\alpha$ -amilase, gamma-glutamyltransferase, and alkaline phosphatase in blood of Wistar rats after cis-platinum administration

GGT has been found to be an essential component of the metabolic activation of alkylating cytostatics which form glutathione-conjugates that are hydrolyzed to cysteinyl-glycine-conjugates by GGT. The cysteine-conjugates are taken up into the cell and converted to a reactive thiol by either cysteine-conjugate  $\beta$ -lyase or S-oxidase. Studies of the nephrotoxic halogenated cytostatics, show that  $< 0.01\%$  of the drug is metabolized to the toxic form (Hannigan, 2001).

Gamma-glutamyl transferase (GGT), is an enzyme that is compared with ALP levels to distinguish between skeletal disease and liver disease. Because GGT is not increased in bone disorders, as is ALP, a normal GGT with an elevated ALP would indicate bone

disease (Figure 3). Other way, because the GGT is more specifically related to the liver, an elevated GGT with an elevated ALP would strengthen the diagnosis of liver or bile-duct disease (van't Sant, 1984).



**Fig. 3.** Concentration of gamma-glutamyltransferase in blood of Wistar rats after cis-platinum administration

### Conclusions

Studies regarding concentration of some serum enzymes from laboratory animals are very important for defining the influence of chemotherapy on normal health status. In case of ALT and AST, we can observe an elevation of both serum enzymes level, some even significant, in experimental groups compared to control groups. Administration of cis-platinum in Wistar rats is characterized by increase values of serum amylase, alkaline phosphatase and gamma-glutamyltransferase directly proportional with administrated doses.

The concentration of serum enzymes in Wistar rats are increased after cis-platinum administration maybe because toxic effects of cis-platinum, in this case hepatotoxic effects. Serum enzymes are implicated in biochemical homeostasis, which is very important for the normal development of physiological processes.

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