

Effect of cisplatin on lipid peroxidation in the whole blood and plasma of female rats

Research paper

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Abstract **Aim:** Cisplatin is known to induce oxidative stress and accumulation of reactive oxygen species (ROS), which are the primary causes of its undesirable side effects. Lipids are a major target of ROS undergoing oxidation upon interaction. Lipid peroxidation products are unstable and degrade into reactive compounds that can damage various biomolecules. Induction of oxidative stress and ROS formation is considered another mechanism of action of cisplatin. This article aims to investigate the effect of cisplatin on lipid peroxidation and the activity of the antioxidant enzyme catalase in whole blood and plasma.

Materials and Methods: The amount of lipid peroxidation products in blood and plasma of rats was determined by absorption of monochromatic ultraviolet light following extraction with a heptane-isopropyl alcohol mixture. The oxidative stress marker malondialdehyde (MDA) in blood and plasma was quantified using the thiobarbituric acid assay. Catalase activity in blood plasma of female rats was assessed using the ammonium molybdate method.

Results: The data show that cisplatin induces significant changes in the levels of lipid peroxidation products, including conjugated dienes and trienes, in both whole blood and plasma of female rats. The oxidation index values calculated for lipid peroxidation products increased to varying degrees following cisplatin exposure. A dramatic increase in MDA concentration was observed in both whole blood and plasma of rats after exposure to cisplatin. In addition, cisplatin exposure resulted in a 55% reduction in catalase activity.

Conclusions: The results demonstrate that cisplatin promotes oxidative stress, increases lipid peroxidation level, and reduces catalase activity in blood. These findings provide insights into the mechanisms by which cisplatin may exert its anticancer effects while also contributing to its side effects.

Keywords Cisplatin – Lipid peroxidation – Conjugated dienes and trienes –Malondialdehyde – Catalase

ABBREVIATIONS

ROS: reactive oxygen species; **FASN:** fatty acid synthetase

INTRODUCTION

Cisplatin (cis-diaminedichloroplatinum [II]) is an antineoplastic drug widely used in chemotherapy to treat various human cancers (Aldossary, 2019; Dasari et al., 2022; Tchounwou et al., 2021). However, its use is limited due to the side effects it causes, especially various toxicities (Aldossary, 2019; Dasari et al., 2022; Tchounwou et al., 2021).

The mechanisms underlying cisplatin-induced toxicities are not fully understood, but studies suggest that these side effects are linked to oxidative stress resulting from the accumulation of reactive oxygen species (ROS) (Aldossary, 2019; Dasari et al., 2022; Tchounwou et al., 2021). ROS can

interact with DNA, lipids, and proteins, leading to lipid peroxidation (LPO) and DNA damage (Gentile et al., 2017; Habtemariam, 2019; Singh & Manna, 2022). Since DNA is the primary target of cisplatin, its damage by ROS could lead to irreversible alterations in DNA, preventing cell division, DNA synthesis and repair, ultimately triggering apoptosis (Dasari et al., 2022; Gentile et al., 2017). However, induction of oxidative stress and ROS formation is considered another mechanism of action of cisplatin (Aldossary, 2019; Habtemariam, 2019; Yang H, 2018). Cisplatin increases the production of free oxygen radicals and decreases antioxidant levels, disrupting the oxidant/antioxidant balance (Habtemariam, 2019; Singh & Manna, 2022).

It is well known that ROS are unstable and highly reactive byproducts of cellular metabolic processes (Gaschler & Stockwell, 2017; Singh & Manna, 2022; Valgimigli, 2023).

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Under normal physiological conditions, ROS are a natural consequence of oxygen metabolism and play a key role in various signaling pathways and homeostasis (Gaschler & Stockwell, 2017; Singh & Manna, 2022). However, excessive ROS accumulation usually occurs due to an imbalance between ROS production and elimination. This imbalance arises from an increased production of oxidants or decreased level of antioxidants or both (Habtemariam, 2019; Gaschler & Stockwell, 2017; Singh & Manna, 2022).

Disturbed redox homeostasis leads to oxidative damage of biomolecules such as proteins, lipids, and nucleic acids, which results in harmful effects on cells. In contrast, modulation of ROS levels contributes to the regulation of cell survival, death, differentiation, and proliferation (Habtemariam, 2019; Gaschler & Stockwell, 2017; Singh & Manna, 2022). Lipids are the primary targets of ROS, which are oxidized upon interaction with oxidants. This process results in the formation of lipoperoxyl radicals and lipid hydroperoxides (Gaschler & Stockwell, 2017; Hauck & Bernlohr, 2016). As highly reactive compounds, lipid peroxides can further propagate ROS generation or degrade into reactive compounds that are capable of cross-linking DNA and proteins. Lipid hydroperoxides have been recognized as key mediators of cellular disease and death (Gaschler & Stockwell, 2017; Gentile et al., 2017; Habtemariam, 2019; Singh & Manna, 2022). LPO products are involved in the intracellular signaling mechanisms that determine a cell's ultimate fate (Gentile et al., 2017; Habtemariam, 2019; Valgimigli, 2023).

The products of LPO subsequently decompose to form reactive aldehydes such as malondialdehyde (MDA), 4-hydroxy-2-nonenal, acrolein, crotonaldehyde, and methylglyoxal. Lipid-derived aldehydes are significant because they can successfully bind to DNA, leading to adduct formation and promoting mutagenic effects (Cui et al., 2018; Hauck & Bernlohr, 2016; Jadoon & Malik, 2017).

Free radicals, produced during LPO, have a very short lifespan and can only produce localized effects. In contrast, aldehydes are more stable and can be transported through the bloodstream or diffuse from their sites of formation to react with various macromolecules (Hauck & Bernlohr, 2016). Due to their ability to react with nucleophilic functional groups in lipids, proteins, and DNA, aldehydes have been identified as “secondary messengers of oxidative stress” (Gentile et al., 2017; Hauck & Bernlohr, 2016).

These aldehydes often attack the free $-NH_2$ - groups of proteins and DNA bases, forming covalent adducts that contribute to the biological consequences of LPO in both normal physiology and pathophysiology (Gentile et al., 2017; Jadoon & Malik, 2017). Among the aldehydes resulting from LPO, MDA is the most extensively studied and has been used as a biomarker of oxidative stress (Cui et al., 2018; Hauck & Bernlohr, 2016; Mirzaei et al., 2021).

Our early research focused on the quantitative changes in MDA levels in certain rat tissues after separate and combined exposure to cisplatin and steroids (Yavroyan et al., 2019). The

aim of this study is to investigate the characteristics of LPO in whole blood and plasma following 24 h of cisplatin exposure.

MATERIALS AND METHODS

Animals

Experiments were conducted in accordance with the “International Recommendations on Carrying out of Biomedical Researches with use of Animals” (CIOMS, 1985; 2016), the “Human Rights and Biomedicine the Oviedo Convention” (CE, 1997), and the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes (CE, 2005) and were approved by the National Center of Bioethics (Armenia).

The study was performed on adult female albino rats (120–150 g weight). The rats were housed in cages under standard laboratory conditions with a 12-h light/12-h dark cycle. All rats had free access to a standard rodent diet and water.

Experimental design

The animals were divided into two groups, each consisting of four rats ($n = 4$). Group 1 served as a control group and received no treatment ($n = 4$). Group 2 received a single dose of cisplatin (8 mg/kg, Sigma-Aldrich, USA) via peritoneal injection. The exposure time for cisplatin was 24 h. All animals were euthanized by decapitation after they were given inhalation anesthesia with chloroform.

Blood sampling and separation of plasma from animals of experimental groups

To assess the LPO level, whole blood of rats and plasma derived from it were used. Blood was collected during decapitation into tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. Four 0.1 ml samples of blood from each animal were taken for extraction of LPO products, and four 0.5 ml samples were taken for MDA quantification. The remaining blood was used for plasma extraction. To obtain plasma, an equal volume of 0.9% sodium chloride (NaCl) solution was added to the remaining blood. After incubation for 15 min at 4°C, the samples were centrifuged at 3000 rpm/min for 10 min using a refrigerated centrifuge (Sigma 3-18K, Germany). The supernatant, which contained plasma, was carefully transferred to clean test tubes and immediately used for LPO product extraction or stored at 2–8°C. Three to four 0.1 ml plasma samples were collected for LPO product extraction, and three to four 1 ml plasma samples were used to measure MDA concentration.

Extraction and determination of LPO products

LPO products were measured using the method developed by Volchegorsky et al. (1989). This method is based on

determining the content of LPO products in biological materials by measuring the absorption of monochromatic light in the ultraviolet spectrum after extracting the lipids with a heptane–isopropyl alcohol mixture. To that end, 0.5 ml of whole blood or 0.1 ml of plasma was mixed with 5 ml of heptane–isopropyl alcohol mixture (1:1 by volume), and the lipids were extracted by shaking for 15 min. Then 5 ml of a heptane–isopropyl alcohol mixture (3:7 by volume) was added to dilute lipid extracts. This step was performed to optimize the optical density values in both phases of the extract. Two milliliters of hydrochloric acid (0.01 N) was added to the diluted lipid extracts to separate the phases and remove non-lipid impurities. After intensive shaking, the samples were incubated for 15 min at 4°C for phase separation. The heptane phase was then transferred to a clean vial, and 1 g of NaCl was added to the water–alcohol phase to dehydrate the isopropyl alcohol extract. After shaking and a 10-min incubation, the phases were separated, and the isopropyl alcohol phase was transferred to a new clean test tube. The heptane phase contains mainly neutral lipids, while the isopropyl alcohol phase contains phospholipids. This technique allows identification of lipoperoxidation products such as conjugated dienes and conjugated trienes in different lipid classes. The optical density of each phase was measured against the corresponding control. Control samples were prepared by following the same extraction procedure, but instead of blood or plasma, 0.5 ml of 0.1% EDTA solution in 0.9% NaCl was used.

The measurements were performed with a Chinese-made BK-UV 1800 Spectrophotometer (Biobase). Absorbance was measured at 220 nm (for double bonds in unsaturated fatty acids), 232 nm (to detect conjugated dienes), and 278 nm (for conjugated trienes). The LPO product content was expressed in conventional units per 1 ml of blood or plasma.

After measuring the optical densities at appropriate wavelengths, both the quantity of LPO products (expressed in conventional units) and the oxidation index were calculated. The oxidation index was determined as the ratio of optical densities at appropriate 232 and 278 nm to the absorption at 220 nm (i.e., D_{232}/D_{220} and D_{278}/D_{220}) (Volchegorsky et al., 1989).

MDA quantification

MDA levels in blood and plasma were measured using the method described by Uchiyama & Mihara (1978). This method is based on the condensation reaction between two molecules of thiobarbituric acid and one molecule of MDA, with the reaction rate depending on temperature, pH, and thiobarbituric acid concentration. The reaction was conducted in an acidic solution at 100°C for 20 min, during which most of the MDA is formed through decomposition of LPO products. MDA reacts with thiobarbituric acid to form a pink-colored complex that absorbs at 532 nm. The biological samples were heated with thiobarbituric acid reagent for 20 min in a boiling water bath. The reaction

mixture contained 30% trichloroacetic acid, 5 N HCl, 0.8% thiobarbituric acid solution, and biological sample. After cooling, the solution was centrifuged at 3000 rpm/min for 10 min and the precipitate obtained was removed (Uchiyama & Mihara, 1978). Absorbance of the supernatant was measured at 532 nm against a blank that contained all reagents without the biological sample. The MDA concentration (in nmol/ml of blood or plasma) was calculated using the appropriate formula (Uchiyama & Mihara, 1978).

Catalase enzyme activity assay

Catalase (EC 1.11.1.6) activity in blood plasma of female rats was measured using the method described by Koroliuk et al. (1988). This method is based on the rate of hydrogen peroxide (H_2O_2) degradation by catalase. The enzyme activity was determined by measuring decrease in H_2O_2 concentration at 410 nm. The method involves the formation of a stable blue complex through the reaction of ammonium molybdate with H_2O_2 , which is then measured photometrically. The enzyme activity was expressed in $\mu\text{mol } H_2O_2/\text{min mg protein}$ (Koroliuk et al., 1988). The protein concentration was determined by the spectrophotometric method described by Kalb & Bernlohr (1977).

Statistical analysis

The data were analyzed using the Excel program and expressed as mean $\pm$ standard deviation ($M \pm SD$) from four independent experiments. Statistical differences between the groups (control and cisplatin-treated groups) were assessed using Student's *t*-test. A *p*-value of <0.05 was considered statistically significant (*).

RESULTS

Since unsaturated fatty acids are the precursors for LPO, our research began with their quantitative estimation in heptane and isopropyl alcohol phases, which were extracted from the whole blood and plasma of female rats in both the control group and after 24 h of cisplatin exposure. As previously mentioned, heptane dissolves neutral lipids, while isopropyl alcohol primarily dissolves phospholipids (Volchegorsky et al., 1989).

The obtained data are presented in Table 1. Results of the quantitative analysis of unsaturated fatty acids indicate that there was no significant change in the heptane phase from plasma following cisplatin exposure. However, a 10% increase in the quantity of unsaturated fatty acids was observed in the heptane phase from whole blood (Table 1). Meanwhile, the quantity of unsaturated fatty acids in the isopropyl alcohol phase increased by 70% in whole blood and 26% in plasma after cisplatin exposure (Table 2).

The quantitative evaluation of conjugated dienes is shown in Tables 1 and 2. Under cisplatin treatment, there was a

Table 1. The amount of lipid peroxidation products (in conventional units/1 ml of blood or plasma) in heptane phase extracted from the whole blood and plasma of rats of the control group and animals injected with cisplatin

Lipid peroxidation products	Heptane phase			
	Whole blood		Blood plasma	
	Baseline without treatment	After cisplatin action	Baseline without treatment	After cisplatin action
Unsaturated fatty acids	41.00 ± 1.64	45.00 ± 1.15	29.27 ± 1.20	27.41 ± 0.78
Conjugated dienes	4.52 ± 0.13	7.15 ± 0.10*	6.44 ± 0.35	8.84 ± 0.14*
Conjugated trienes	1.63 ± 0.10	3.27 ± 0.16*	2.45 ± 0.15	4.00 ± 0.11*

Statistical significance between baseline and experimental group * $P < 0.05$

notable increase in the quantity of conjugated dienes in both the heptane and isopropyl alcohol phases from the whole blood and plasma of female rats (Tables 1 and 2). The amount of conjugated dienes in the heptane phase extracted from the whole blood increased by 58% and in blood plasma, it increased by 37% following cisplatin treatment (Table 1). In the isopropyl alcohol phase, cisplatin induced a 93% increase in conjugated dienes in whole blood and a 26% increase in plasma (Table 2).

Cisplatin thus stimulated the LPO process, affecting the quantity of conjugated dienes, which are primary products of lipids peroxidation. The quantitative analysis of conjugated trienes extracted from whole blood and plasma of female rats is also presented in Tables 1 and 2.

The obtained data indicate that, after cisplatin exposure, the quantity of conjugated trienes in the heptane phases of whole blood and plasma increased by 100.6% and 63%, respectively, compared to baseline (Table 1). In the isopropyl alcohol phases of whole blood and plasma, the amount of conjugated trienes increased by 60% and 24%, respectively, after cisplatin treatment (Table 2). This suggests that cisplatin, by stimulating LPO, increased the amount of conjugated trienes in both heptane phase (which contains neutral lipids) and isopropyl alcohol phase (which contains phospholipids), in both whole blood and plasma.

Based on the quantitative assessment of LPO products in whole blood and plasma, we calculated the oxidation index for conjugated dienes and trienes in the control and cisplatin-treated groups (Table 3). The calculated oxidation index values for different LPO products showed varying degrees of increase following cisplatin exposure (Table 3).

No change in the oxidation index value for conjugated trienes was observed in isopropyl alcohol phases isolated from whole blood and plasma after cisplatin exposure. In addition, cisplatin did not affect the oxidation index value for conjugated dienes in the isopropyl alcohol phase extracted from rat blood plasma (Table 3). In contrast, in the remaining phases, exposure to cisplatin resulted in an increase in oxidation index to varying extents compared to baseline (Table 3).

No change in the oxidation index value for conjugated trienes was observed in isopropyl alcohol phases isolated from rat whole blood and plasma after cisplatin exposure. In addition, cisplatin did not affect the oxidation index value for conjugated dienes in the isopropyl alcohol phase extracted from rat blood plasma (Table 3). In contrast, in the remaining phases, exposure to cisplatin resulted in an increase in the oxidation index to varying extents compared to baseline (Table 3).

Specifically, the oxidation index values for conjugated dienes in the heptane phases obtained from both whole blood and plasma increased by 44% and 47%, respectively, compared to baseline. In the isopropyl alcohol phase of whole blood, the oxidation index for conjugated dienes increased by only 14%, while no change was observed in the oxidation index for conjugated trienes (Table 3). Following cisplatin exposure, the oxidation index values for conjugated trienes in the heptane phase from both whole blood and plasma increased by 83% and 74%, respectively (Table 3).

The next part of our research focused on the quantification of MDA. The data obtained showed that the MDA concentration in whole blood in the control group was approximately 1.4 times higher than in plasma (Table 4). After 24 h of cisplatin exposure, the MDA levels increased significantly, rising by 144% in whole blood and 58% in plasma (Table 4).

In addition, we investigated the activity of the antioxidant enzyme catalase in the blood plasma of female rats. The results indicated that cisplatin exposure for 24 h led to a significant reduction in catalase activity in the plasma. Specifically, cisplatin decreased catalase activity by 55% compared to baseline levels (Table 4).

DISCUSSION

The intensity of LPO depends on the presence of free radicals in the cell. It is known that the level of LPO is determined by the quantity of products formed as a result of this process (Gaschler & Stockwell, 2017; Hauck & Bernlohr, 2016; Valgimigli, 2023).

Unsaturated fatty acids, both free and incorporated into various lipids, serve as substrates for LPO (Gaschler &

Table 2. The amount of lipid peroxidation products (in conventional units/1 ml of blood or plasma) in isopropyl alcohol phase extracted from the whole blood and plasma of rats of control group and animals injected with cisplatin.

Lipid peroxidation products	Isopropyl alcohol phase			
	Whole blood		Blood plasma	
	Baseline without treatment	After cisplatin action	Baseline without treatment	After cisplatin action
Unsaturated fatty acids	33.05 ± 0.58	56.00 ± 2.40*	22.00 ± 1.30	27.75 ± 0.23*
Conjugated dienes	9.00 ± 0.20	17.38 ± 0.34*	6.88 ± 0.25	8.70 ± 0.31*
Conjugated trienes	7.58 ± 0.14	12.10 ± 0.30*	3.54 ± 0.05	4.40 ± 0.20*

Statistical significance between baseline and experimental group *P < 0.05.

Table 3. Oxidation index for lipid peroxidation products, conjugated dienes and trienes, from the heptane and isopropyl alcohol phases of whole blood and plasma at baseline and after 24-h exposure to cisplatin.

Oxidation index	Phases of lipid peroxidation products	Whole blood		Blood plasma	
		Baseline without treatment	After cisplatin action	Baseline without treatment	After cisplatin action
For conjugated dienes (D_{232}/D_{220})	Heptane phase	0.110	0.158	0.220	0.323
	Isopropyl alcohol phase	0.272	0.310	0.313	0.314
For conjugated trienes (D_{278}/D_{220})	Heptane phase	0.040	0.073	0.084	0.146
	Isopropyl alcohol phase	0.230	0.216	0.161	0.159

Table 4. Changes in concentration of malondialdehyde and of catalase enzyme activity in blood of female rats.

Concentration of malondialdehyde (nmol/ml)		
	Baseline without treatment	After cisplatin action
Whole blood	6.62 ± 0.32	16.30 ± 0.70*
Blood plasma	4.62 ± 0.22	7.31 ± 0.25*
Activity of catalase enzyme in $\mu\text{mol of H}_2\text{O}_2/\text{mg protein min}$		
Blood plasma	517.00 ± 8.50	233.00 ± 4.60*

Statistical significance between baseline and experimental group *P < 0.05.

Stockwell, 2017; Hauck & Bernlohr, 2016). The process begins when free radicals attack polyunsaturated fatty acids, leading to the formation of lipid radicals. These lipid radicals can then initiate new radical formation, triggering a chain reaction (Gaschler & Stockwell, 2017; Hauck & Bernlohr, 2016; Valgimigli, 2023). As a result of the attack by ROS, unsaturated fatty acids are oxidized, forming lipoperoxyl radicals and lipid hydroperoxides. The latter undergo intramolecular transformations, converting into unstable compounds such as conjugated dienes and trienes, that can break down further into various aldehydes. One such aldehyde, MDA, has been

well studied and is considered a marker for both oxidative stress and LPO processes (Gaschler & Stockwell, 2017; Hauck & Bernlohr, 2016; Jadoon & Malik, 2017).

This article presents the results of studies investigating the level of oxidative stress and LPO in the whole blood and plasma of rats after exposure to cisplatin. Our findings revealed quantitative changes in unsaturated fatty acids and LPO products (conjugated dienes and trienes) following cisplatin exposure. Specifically, after 24 h of cisplatin treatment, we observed quantitative changes in unsaturated fatty acids in both the heptane and isopropyl alcohol phases extracted from rat whole blood. In the case of plasma, cisplatin exposure resulted in changes only in the isopropyl alcohol phase (containing phospholipids), with no changes recorded in the heptane phase (Tables 1 and 2). In all cases where changes were observed, an increase in the quantity of unsaturated fatty acids was recorded.

It is well known that metabolic changes occur in cancer cells to support the growth of tumors (Jin et al., 2023; Plathow & Weber, 2008). Lipid metabolic reprogramming is an emerging hallmark of cancer (Jin et al., 2023). One key metabolic change is the activation of fatty acid synthesis, resulting from the overexpression of fatty acid synthetase (FASN) enzyme (Jin et al., 2023; Plathow & Weber, 2008). Overexpression of FASN, a multi-enzyme protein, has become a target for cancer treatment strategies. FASN is not only essential for the *de novo*

synthesis of long-chain fatty acids, but also plays a key role in the synthesis of all lipids (Jin et al., 2023; Plathow & Weber, 2008). Cancer cells rely on this process to meet their increased demands for membrane production, energy, and protein synthesis (Jin et al., 2023; Plathow & Weber, 2008). Furthermore, FASN enzyme activity holds diagnostic significance in the clinic (Jin et al., 2023). FASN is a target for many anticancer drugs, including cisplatin (Jin et al., 2023; Plathow & Weber, 2008). Cisplatin, a widely used chemotherapy drug, targets FASN, suppressing its overexpression and, as a result, the synthesis of unsaturated fatty acids (Garcia J, 2013; Jin et al., 2023; Plathow & Weber, 2008).

It has been shown that cisplatin not only suppresses lipid synthesis or lipogenesis, but also promotes lipid breakdown, releasing free unsaturated fatty acids (Garcia, 2013; Plathow & Weber, 2008).

The observed increase in unsaturated fatty acids in some of investigated phases does not contradict cisplatin's metabolic suppressive effects, but instead suggests the lipolytic properties of this drug.

Changes in unsaturated fatty acid levels likely reflect the cleavage of various lipids (Garcia, 2013; Plathow & Weber 2008). In instances where there was little or no change in the quantity of unsaturated fatty acids, the increase in conjugated dienes level after cisplatin exposure may explain the observation. The data show that cisplatin treatment led to an increase in the quantity of conjugated dienes to varying degrees in both heptane and isopropyl alcohol phases extracted from the whole blood and plasma of rats (Tables 1 and 2).

The statistically significant increase in conjugated dienes suggests that cisplatin promotes LPO (Cui et al., 2018). Other studies have also reported increased LPO in response to cisplatin (Cui et al., 2018).

Activation of LPO after cisplatin injection was further supported by the oxidation index calculations for conjugated dienes. The oxidation index values increased in the heptane phases extracted from whole blood and plasma (Table 3). In contrast, the oxidation index for conjugated dienes in the isopropyl alcohol phase, containing phospholipids, increased only in the phase extracted from whole blood. No significant changes were observed in the oxidation index for conjugated trienes in the isopropyl alcohol phases extracted from either whole blood or plasma (Table 3). The unchanged oxidation index values for conjugated dienes and trienes in certain phases, particularly in whole blood and plasma, may be due to a proportional increase in the amounts of unsaturated fatty acids, conjugated dienes, and trienes as a result of cisplatin exposure (Tables 2 and 3).

Our quantitative analysis confirmed that the levels of conjugated trienes in both heptane and isopropyl alcohol phases extracted from whole blood and plasma significantly increased after cisplatin treatment compared to baseline levels (Tables 1 and 2). Cisplatin treatment resulted in increased

amounts of conjugated dienes and trienes in both heptane (containing neutral lipids) and isopropyl alcohol (containing phospholipids) phases extracted from whole blood and plasma. Since conjugated dienes and trienes are products of LPO, increase in their levels under the influence of cisplatin strongly suggests that the drug stimulates LPO. To further confirm this, we also measured the amount of MDA, a widely used marker for LPO, in whole blood and plasma. Although MDA is not highly specific as it can also be formed from non-lipid molecules such as proteins, bile pigments, nucleic acids, and carbohydrates, it remains a widely accepted indicator of LPO (Cui et al., 2018; Gentile, 2017; Jadoon & Malik, 2017). Some researchers suggest that conjugated dienes may be a more accurate marker of LPO, as they are primary products of this process (Cui et al., 2018).

Our results showed a dramatic increase in MDA levels, both in whole blood (by 144%) and plasma (by 58%) after 24 h of cisplatin exposure (Table 4). Other studies have reported similar increases in MDA levels following cisplatin treatment, including a 244% increase in blood and an 85% increase in plasma (Gaschler & Stockwell, 2017; Hauck & Bernlohr, 2016), as well as a 59% increase in plasma (Singh & Manna, 2022; Tchounwou et al., 2021). Cisplatin has been shown to significantly elevate MDA levels in a dose-dependent manner (Singh & Manna, 2022; Tchounwou et al., 2021). Despite some controversy, MDA continues to be widely regarded as a reliable marker of LPO (Cui et al., 2018; Hauck & Bernlohr, 2016; Jadoon & Malik, 2017).

It is well known that the main components of the antioxidant defense system are enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, which metabolize intracellular ROS and maintain cellular homeostasis (Cui et al., 2018; Hauck & Bernlohr, 2016; Valgimigli, 2023). One key antioxidant enzyme is catalase, whose activity we measured in the blood plasma of rats following cisplatin exposure. As expected, cisplatin decreased the catalase activity by 55% in blood plasma compared to baseline level (Table 4).

Suppression of catalase activity after cisplatin exposure suggests that this anticancer drug promotes oxidative stress through inhibition of antioxidant enzyme function, thereby contributing to an increase in LPO. This inhibition of catalase activity highlights the mechanism by which cisplatin stimulates oxidative stress and LPO. However, further studies are needed to better understand the full impact of cisplatin on LPO processes.

CONCLUSIONS

In summary, the results of this study indicate that 24-h exposure to the anticancer drug cisplatin leads to significant changes in the levels of LPO products in both whole blood and plasma. Notably, cisplatin dramatically increased the amount of MDA, a final product of LPO. In addition, exposure to cisplatin resulted in a decrease in the activity of the

antioxidant enzyme catalase. These quantitative changes suggest the activation of LPO processes as a consequence of cisplatin-induced oxidative stress.

Findings of this study further confirm the prooxidant nature of the anticancer drug cisplatin. The observed changes in the levels of conjugated dienes and trienes, as well as their oxidation index values, indicate that cisplatin induces oxidative stress and activates LPO. These results provide

insights into the potential mechanisms through which cisplatin exerts its anticancer effects. However, further studies are needed to gain a deeper understanding of how cisplatin affects LPO processes.

CONFLICT OF INTEREST

None to declare.

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