DETERMINATION OF BIOCHEMICAL INDICATORS WHEN MODELING HIPEC WITH CISPLATIN ON RATS

E. D. Pykhtieieva, A. M. Tretyakov, A. I. Gozhenko, E. G. Pykhtieieva

Ukrainian Research Institute of Transport Medicine, Odessa, Ukraine

Abstract

HIPEC (Hyperthermic intraperitoneal chemotherapy; hyperthermic intraoperative intraperitoneally chemoperfusion) is a method of treating primary and secondary peritoneal tumors. Perfusion of the peritoneal cavity with solutions with cytotoxic agents at a temperature above the physiological norm (41-43 ° C) occurs when HIPEC. The conducted biochemical studies suggest that with HIPEC, elevated temperature is an important factor and leads to potentiation of the toxic effect of cisplatin. Despite the higher accumulation of platinum in the kidneys during cold solution perfusion, the resulting change in biochemical parameters is higher with the simultaneous action of elevated temperature and cisplatin.

Key words: rats, cisplatin, temperature, acid phosphatase, lipid peroxidation, creatinine.

HIPEC (Hyperthermic intraperitoneal chemotherapy; hyperthermic intraoperative intraperitoneally chemoperfusion) is a method of treating primary and secondary peritoneal tumors. Perfusion of the peritoneal cavity with solutions with cytotoxic agents at a temperature above the physiological norm (41-43 ° C) occurs when HIPEC [1, 2]. The duration of the procedure (30-90 minutes) ensures maximum efficacy of the chemotherapy drug with the least possible toxic effect. The pathophysiological mechanisms of the side effects of cisplatin in HIPEC are not well understood, which makes our work relevant.

Materials and Methods: The HIPEC modeling and intraperitoneal chemoperfusion experiment at normal temperature was conducted on healthy male rats (obtained from the vivarium of Odessa National Medical University) weighing 180-200 g. The total dose of cisplatin (CP) was 4 mg/kg (0.72 - 0.80 mg per animal). The modeling technique is described in detail by us earlier. [3].

The state of free radical oxidation, formation of ACF and the development of oxidative stress was determined by the intensity of spontaneous accumulation of low molecular weight lipid peroxidation products that react with thiobarbituric acid to form malondialdehyde - TBA-active products in the blood, tissue homogenates of the organs studied [4]. In addition, the activity of key cellular metabolism enzymes was determined: acid phosphatase (AP, 3.1.3.2), superoxide dismutase (SOD, 1.5.1.5) [5]. The AP activity was determined using S-glycerophosphate sodium as a substrate; under the action of phosphatases, it undergoes hydrolysis with the release of inorganic phosphorus. The determination was carried out in a buffer solution at a pH of 4.5-5.0. The protein concentration was determined using the Folin-Chiocalteu reagent [6]. The plasma creatinine concentration was determined using biochemical kits manufactured by Felicity Diagnostics, Dnipro, Ukraine.

Results

Despite the relatively short exposure time when modeling HIPEC, a sufficiently high effective dose of cisplatin in combination with elevated temperature leads to moderately pronounced changes in the biochemical parameters of experimental animals compared to control ones.

So, in control animals, serum creatinine was 48.4 ± 6.2 mmol/l. After perfusion with a cold solution of CP, the creatinine content increased by 19%, and after HIPEC - by almost 54% compared with the control (Fig. 1).

At the same time, the content of acid phosphatase (AP) in the serum was significantly ($p < 0.05$ compared with the control for both experimental groups) (Fig. 2), while the changes between the experimental groups were not significant ($p > 0.05$). It is known that AP is found in the prostate gland, liver, erythrocytes, platelets, kidneys, spleen and other tissues. With the defeat of these tissues, the activity of KF in the serum increases [7].The accumulation of platinum in the tissues of the kidney, liver and spleen, which we found earlier, suggests that the increase in serum CF activity is caused by the cytotoxic effect of cisplatin on target organs.
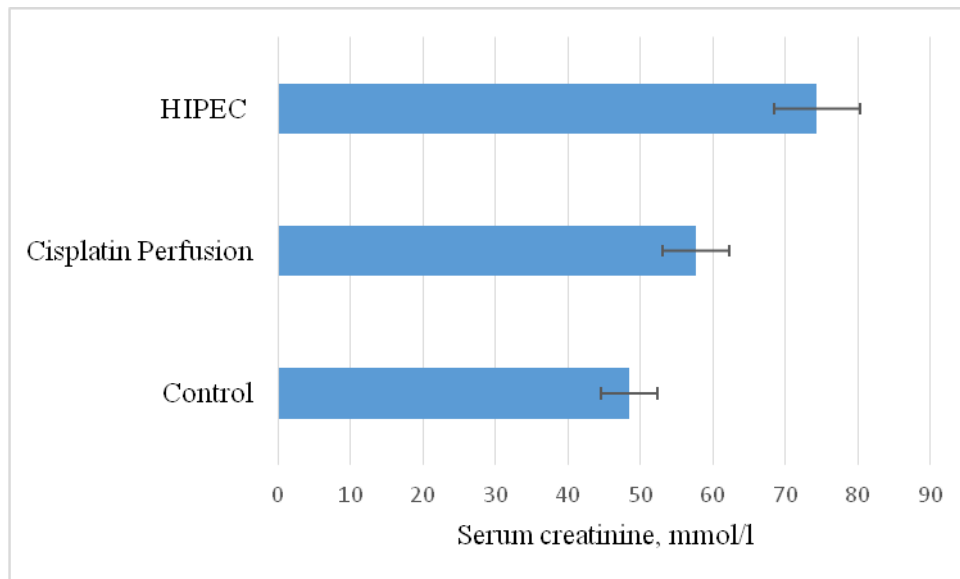
Fig. 1. Changes in the content of serum creatinine after perfusing a solution of CP at 20 ° C and HIPEC, n = 7, exposure time 60 min.
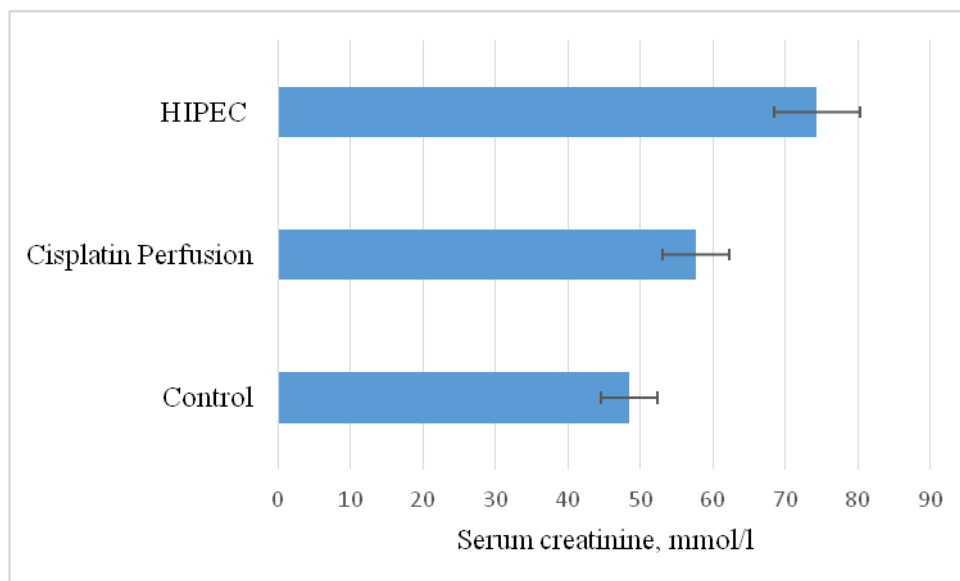
Fig. 2. Changes in activity of serum AP after perfusing a solution of CP at 20 ° C and HIPEC, n = 7, exposure time 60 min.

There was also a compensatory increase in the activity of the main antioxidant enzymes in the tissues of the liver and kidneys. SOD activity increased in the liver by 18.4% and 26.9%, and in the kidneys by 19.6 and 27.7% (with cold solution and HIPEC, respectively). Despite this, we observed an increase in the TBC of active products of lipid peroxidation on average in the liver by 12.7 and 18.9% (respectively, for cold solution and

HIPEC perfusion) and the kidneys by an average of 19.7% and 24.1% (respectively for cold solution perfusion and HIPEC) (Fig. 3).

Rybak LP [8]in 2007, it was shown that cisplatin activates NOX3 (NADPH oxidase 3 (NOX3), an oxidoreductase of the cell membrane that forms superoxide radicals when an electron transitions from NADP to oxygen). This leads to an increase in superoxide production and the formation of hydrogen peroxide, which can turn into free hydroxyl radicals. These radicals are extremely active and can react with polyunsaturated fatty acids in membranes to form 4-hydroxynenal (extremely toxic aldehyde). Thus, GAOS and antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase) are activated to minimize ROS overload in cells [9, 10].

The formation of an excessive amount of reactive oxygen species (ROS) in the reaction of lipid peroxidation (LPO) plays a leading role in the pathogenesis of the toxic effects of heavy metals and is one of the causes of changes in the structure and function of biological membranes. [11]. At the same time, LPO is a normal metabolic process, occurs in almost all organs and tissues of the body. To study the processes of lipid peroxidation in the tissues of the liver, kidneys and blood, malonic dialdehyde was determined using thiobarbituric acid.

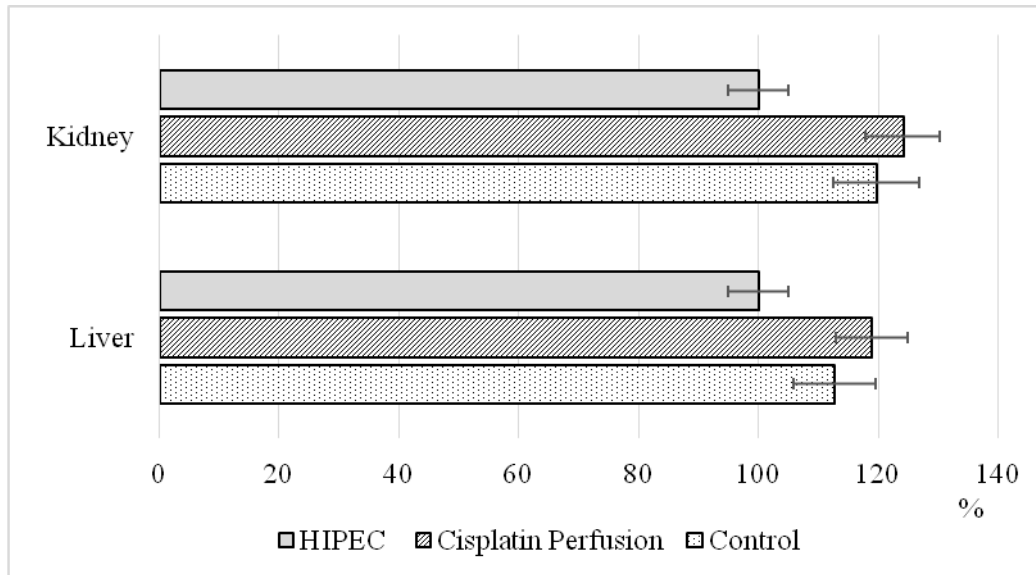Fig. 3. The relative content of malondialdehyde in the liver and kidneys tissues after perfusing a solution of CP at 20 ° C and HIPEC, n = 7, exposure time 60 min. (in % of the control).

At the same time, activation of the lysosomal marker enzyme AP in the tissues of the liver and kidneys occurs. This is probably a marker of activation and increase in the number

of lysosomes when exposed to cisplatin (Fig. 4). The mechanism for removing platinum from the cell has not been finally clarified to date, although it is known that platinum remains in the form of a complex compound when implementing its therapeutic action. Due to this, it bifunctionally alkylates DNA strands, suppresses the biosynthesis of nucleic acids, causes cell death. Platinum complexes with cis-arrangement of halogen atoms can form stable chelates with purine and pyrimidine components of nucleic acid molecules and thus form bonds within one strand or parallel strands of the DNA double helix. The blockage of DNA strands is maintained for several days after cisplatin administration. But the further fate of the CPU is less studied. Probably (and this confirms the increase in the activity of lysosomal enzymes) in the removal of CP from those cells that did not die, lysosomes are involved. The involvement of lysosomes in the removal of other heavy metals has been shown previously [12].The authors pointed out that concrete systems (cellular and metabolic) that provide for the delivery of metals to lysosomes and the transformation of metal-protein and metal-nucleic complexes in lysosomes have not been studied much today. The mechanisms of intracellular transfer and removal of metals from lysosomes into the cytosol and extracellular environment are practically unknown.
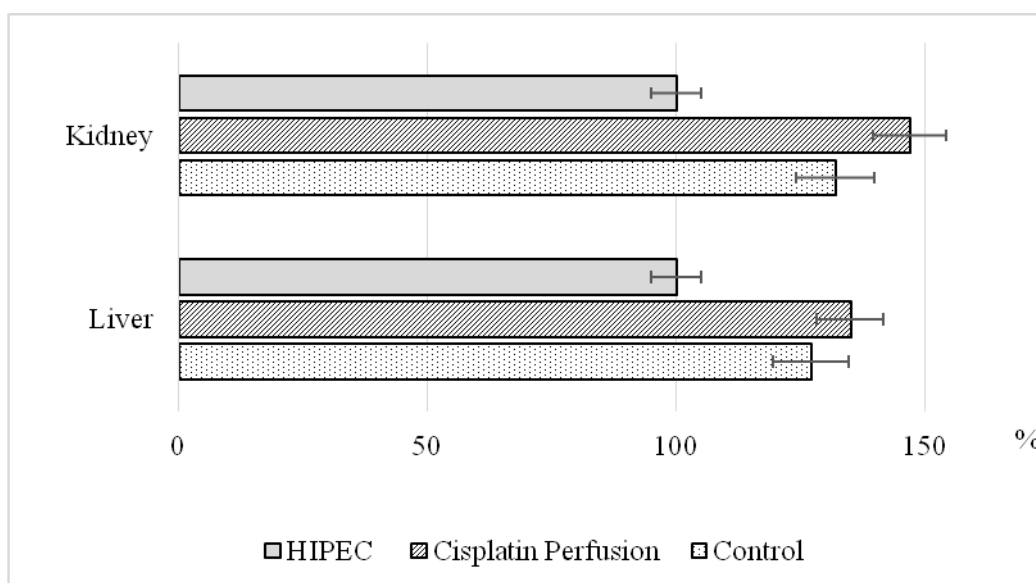

Fig. 4. The relative activity of AP in 10% homogenate of the liver and kidneys tissues after perfusing a solution of CP at 20 ° C and HIPEC, n = 7, exposure time 60 min. (in % of the control).

Conclusion: the conducted biochemical studies suggest that with HIPEC, elevated temperature is an important factor and leads to potentiation of the toxic effect of CP. Despite the higher accumulation of platinum in the kidneys during cold solution perfusion, the

resulting change in biochemical parameters is higher with the simultaneous action of elevated temperature and CP.

References

[1] Kampinga HH, Dynlacht JR, Dikomey E. Mechanism of radiosensitization by hyperthermia (& gt; or = 43 degrees C) as derived from studies with DNA repair defective mutant cell lines. Int J Hyperthermia. 2004;20:131–139.

[2] Issels RD. Hyperthermia adds to chemotherapy. Eur J Cancer. 2008;44:2546–2554.

[3] Pykhtieieva E.D., Gozhenko A.I., Pykhtieieva E.G., Bolshoy D.V., Tretyakov A.M. Changes of microelement homeostasis when modeling HIPEC with cisplatine. Journal of Education, Health and Sport. 2019, 9 (2), p. 569–578.

[4] Stalnaya I.D., Garishvili T.G. Method for the determination of malonic dialdehyde using thiobarbituric acid. "Modern methods in biochemistry" ed. Orekhovich VN, Moscow: Medicine, 1977. S. 66-68. (in rus.)

[5] Nishikimi N. Determination of SOD activity in the biological substrates/ N. Nishikimi, N.A.Rao, K. Yagi // Biochem. Biophys. Res. Commun. - 1972 – Vol. 46. – № 9. – P. 846-852.

[6] Handbook of biochemistry: translated from English. / Dawson R., Elliot D., Elliot U., Jones K.// M .: Mir, 1991. - 544 p. (in rus.)

[7] Angelski S., Yakubovskiy Z., Dominichak MG Clinical biochemistry. - Sopot: Perseus, 2000. - 453 pp. (in ukr.)

[8] Rybak LP (2007) Mechanisms of cisplatin ototoxicity and progress in otoprotection. Curr Opin Otolaryngol Head Neck Surg 15:364–369

[9] Gonza´lez-Garcı´a JA, Nevado J, Garcı´a-Berrocal JR, Sa´nchezRodrı´guez C, Sanz R, Ramı´rez-Camacho R (2010) Endogenous protection against oxidative stress caused by cisplatin: role of superoxide dismutase. Acta Otolaryngol 130:453–457

[10] Manohar S., Leung N. Cisplatin nephrotoxicity: a review of the literature //Journal of nephrology. – 2018. – Т. 31. – №. 1. – С. 15-25.

[11] Lock E.A. Renal Xenobiotic Metabolism. - In: Comprechensive Toxicology. Vol. 7. Renal Toxicology. – Cambridge, UK: Pergamon Press, 1997. – P.77-98.

[12] Shafran L.M., Bolshoy D.V., Pyhtieieva E. G., Tretyakova E. V. The role of lysosomes in the mechanism of protection and cell damage under the action of heavy metals. Modern Problems of Toxicology - 2004 - No. (3) - P. 17-25. (in rus.)