The Study of the Correlation Between the Level of Antioxidant, Enzyme Activity and Administration Zinc in Oxidative Stress Induced by Cyclophosphamide

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A well designed targeted chemotherapy is considered one of the goals of anticancer treatment, significantly improving the survival rate, but there is evidence suggesting it may presents significant drawbacks. Oxidative stress is frequently associated with chemotherapeutic agents, generating increased reactive oxygen species which may alter the female genital system. There are numerous opinions suggesting supplemental antioxidants in chemotherapy represent a positive therapeutic strategy. This study aim was to assess if antioxidants as zinc are able to preserve female rat's genital system in cyclophosphamide (CP) - induced oxidative stress. In experiment there was used 21 female Wistar rats which were randomly divided in three groups: control, cyclophosphamide - administered group and cyclophosphamide-$ZnCl_2$ administered group. After four weeks, blood was collected to determine malondialdehyde (MDA) level and glutathione peroxidase (GPx) activity. Our results suggests that zinc reduces the deleterious effect of oxidative stress on female rats genital system, the decrease of MDA level together with GPx increase being considered as a result of Zn administration.

Keywords: malondialdehyde, glutathione peroxidase, antioxidants, administered group, therapeutic strategy

Chemotherapy, radiotherapy and surgery represent the main therapeutic resources we can use against different types of cancer. Many chemotherapeutic agents, depending on their structure, are known to promote high levels of oxidative stress [1].

Cyclophosphamide is a cytotoxic drug, an inactive cyclic phosphamide ester of mechlorethamine, enzymatically converted to active alkylating metabolites (hydroxy-cyclophophosphamide, aldophosphamide, acrolein, phosphoramidmustard). Widely used in a broad spectrum of different types of cancers(breast, ovary, lung, Ewing's sarcoma, head and neck cancer, retinoblastoma, multiple myeloma, neuroblastoma, acute lymphoblastic leukemia, Hodgkin's disease or Burkitt's Lymphoma), but also in autoimmune diseases and other pathologic conditions (Behcet disease, systemic lupus erytemathosus, dermatomyositis, paraneoplastic pemphigus). Its antitumoral effect is based on a particular interference with growth and replication of tumor cells [2, 3].

Oxidative stress is a key contributor to the cyclophosphamide toxicity; the increase of reactive oxygen species will generate different types of tissular alterations [4-7]. For this reason, despite his high efficiency in cancer combat, it will induce some limitations of cyclo-phosphamide therapeutic use [8].

The antitumoral effects of cyclophosphamide are correlated with DNA damage, DNA cross-linking being frequently mentioned; the current state of knowledge clearly affirm that the ability of cyclophosphamide to eliminate tumor cells depends on its capacity to neutralize DNA through progressive lesions, avoiding its possibilities to be repaired. All these events will led to the destruction and death of tumor cells [9, 10].

A very important aspect has to be taken into consideration in chemotherapy: the perfectly managed destruction of tumor cells during chemotherapy must not be followed by a similar destruction of normal healthy cells [11, 12].

Considering cancer as a dynamic process, in which the tumor cells profile is dysregulated, early detection and cure is not always possible; in recent years has been widely discussed the proper approach of cancer therapy. It is a general tendency to try to control the disease, relieving the symptoms and prolonging patients life [13, 14]. On the other hand, one might expect that deciphering all the mechanisms involved in pathophysiological conditions would lead to a better way of assuming a new attitude towards cancer therapy, determining cancer outcome [15, 16].

In case of female genital system, one of the long term effects of cyclophosphamide therapy is represented by infertility. The gonadal damage is frequently reported as a distinct irreversible consequence of cyclophosphamide [17]. Major concerns regarding side effects of cyclophosphamide have to face the interference of distinct events, which have not yet completely been revealed. Oxidative stress, through the reactive oxygen species, increses the local damage accumulation, exceeding the tissular ability of restoring the initial cellular status [18].

In this context, the antioxidants administration can promote a consistent defence, improving the efficiency of chemotherapy [19]. Although a great number of studies sustain the positive role of antioxidants in chemotherapy, an inconsistent data was reported related to its impact on female genital system [20].

Our experimental study intends to explore the antioxidant effect of zinc on female genital system of rats in cyclophosphamide –induced oxidative stress, pointing on the activity of MDA and GPx, as distinct markers of oxidative stress.

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Experimental part

Materials and methods

Animal experimental protocol

For this experiment were used 21 female rats form Wistar. The animals were kept in similar conditions 7 days before starting the experiment, in separate cages, under standard laboratory conditions, with standard food and water ad libitum. After this period of acclimatization, the rats were randomly divided in three groups: control group; cyclophosphamide-administered group(CP), 150 mg/kg, twice a week, in intraperitoneally administration; cyclophosphamide + $ZnCl_2$ - administered group (CP + $ZnCl_2$); cyclophosphamide is administrated in a similar manner with group 2, $ZnCl_2$ - 5mg/kg, daily, oral. After 120 days, the blood was prelevated for biochemical exam of MDA and GPx. Isoflurane was administered through inhalation and after the disappearance of vital signs, was followed by cervical dislocation for every rat. Tissular samples were taken for histopathologic exam. The experimental procedures were carried out in accordance with the mandatory principles of the Ethical Committee of the Grigore T. Popa University of Medicine and Pharmacy Iasi [21, 22].

Biochemical assay

Biomarkers of the oxidative stress were determined. MDA was determined based on method of Slater and Sawyer [23]. GPx was spectrofotometrically determined based on method of Paglia and Valentine [24].

Statistical analysis

All results are expressed as mean ± SD. To determine the significance of differences between groups analy-sis of variance (ANOVA) was used. Differences were considered statistically significant when p value < 0.05.

Results and discussions

The estimation of MDA activity as a result of cyclophosphamide-induced oxidative stress revealed an increase reported to control; after $ZnCl_2$ administration, MDA presented a significant decrease (fig. 1).

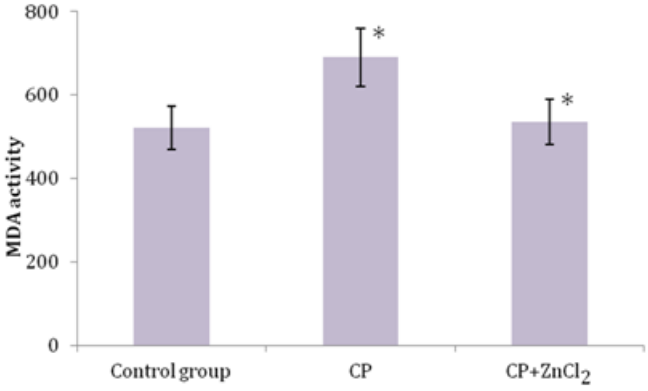

Fig. 1. $ZnCl_2$ effect on MDA level in cyclophosphamide – induced oxidative stress.* p < 0.05 *vs* control

On the other hand, GPx activity was decreased as a result of oxidative stress during cyclophosphamide administration; $ZnCl_2$ determined an increase in GPx activity (fig. 2).

Increasing evidence sustain the fact that the efficiency of chemotherapeutic design involving oxidative stress sequences, as a condition defined by high levels of reactive oxygen species, may be considerably improved by adding antioxidants. Since the administration of cyclo-phosphamide triggers distinct pathways of oxidative stress,

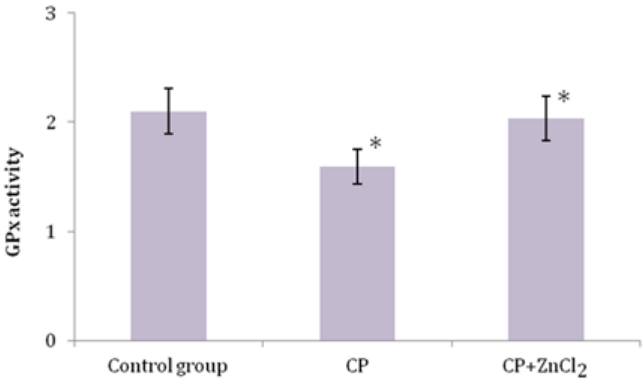

Fig. 2. $ZnCl_2$ effect on GPx activity in cyclophosphamide - induced oxidative stress.* p < 0.05 *vs* control

one could expect an association with certain antioxidants to develop a protective effect [25, 26].

This theory ruled out for numerous systems and organs from the human body; only a little attention was accorded to this theory in case of female genital system. Because of its performances, cyclophosphamide is used in a large spectrum of female genital diseases, although it impairs reproductive function. Gonadal disturbance is one of the major effects of cyclophosphamide toxicity [27, 28].

We may appreciate that antioxidants are of interest [29-32], but there are opinions pleading for their inefficiency, because of a possible interference with therapeutic events and mechanisms, reducing the favourable results of chemotherapy [33, 34].

There is no clear support to antioxidant relevance in chemotherapy involving oxidative stress mechanisms in female genital system. If oxidative damages have been extensively studied, research on the antioxidants interfering chemotherapy and effects in the female reproductive pathology remains still [35, 36].

We developed an experimental animal model to investigate if a beneficial influence of zinc as antioxidant is exerted on female rats genital system when cyclophosphamide – administered.

We pick on zinc because this trace element because to our knowledge, few data approach its proper role on female reproductive system [37, 38]. Zinc has a well defined function in cellular proliferation, with direct impact on factors involved in DNA synthesis. When DNA is damaged during oxidative stress, zinc plays a role in its repair. In the same time, the presence of zinc in one of the most significant members of SOD antioxidant enzyme family, Cu-Zn SOD, makes it a real player in antioxidant defense [39]. Prasad's study sustained MDA decrease under zinc modulation in oxidative stress, with special reference to the age related disorders, but no correlations were done to zinc influence on female genital system [40].

Our study confirms the potential of cyclophosphamide to induce oxidative stress; the indicator of lipid peroxidation, MDA, is significantly increased, suggesting a firm correlation with an increase in protein oxidation parameters in the female genital system [41]. This fact contributes to an accumulation of by products which enhances toxic injuries and emphasizes the cellular alterations [42, 43].

In our study, in parallel with MDA elevation, compared with control, we observed GPx decrease in cyclo-phosphamide group. GPx, considered an important peroxide scavenger enzyme, may have low values as a possible result of glutathione depletion [44, 45]. Miyamoto studies pointed on GPx inactivation in oxidative stress, as a major cause of its decrease, while Chen accorded a particular significance to GPx antioxidant potential in a strong correlation with GSH activity [46, 47].

After $ZnCl_2$ administration, we could observe a improving of the two investigated biomarkers. MDA level becomes compared with that from cyclophosphamide group. A reduced level of MDA usually suggests that the amplitude of the oxidative stress considerably diminished [48, 49]. High activity values of GPX were observed as a result of $ZnCl_2$ administration [50].

Conclusions

Our experimental study sustains the efficiency of antioxidants in chemotherapy, when oxidative stress is triggered as an important contributor mechanism for tissular alterations, pinpointing a relevant activity of two distinct seric biomarkers, MDA and GPx.

We consider zinc as may contribute to reduce the effects of cyclophosphamide-induced oxidative stress on female rats genital system, protection sustained by the evidence of MDA activity decrease and GPx level increase; based on our observation, it turns out that zinc can be used as supplemental antioxidant in chemotherapy targeting female genital system.

Zinc improves the local response to oxidative stress and reduces cyclophosphamide limitations in therapy.

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