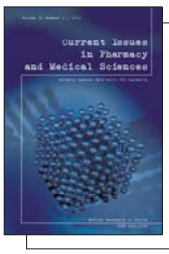

2012 © Curr. Issues Pharm. Med. Sci. Vol. 25, No. 3, Pages 262-265

Current Issues in Pharmacy and Medical Sciences

Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA
on-line: www.umlub.pl/pharmacy

Assessment of oxidative stress parameters in rats pretreated with Ukrain and amitriptyline

MONIKA GAWROŃSKA-GRZYWACZ*, MARIOLA HERBET, MONIKA SZCZĄCHOR, EWA JAGIEŁŁO-WÓJTOWICZ

* Department of Toxicology, Medical University of Lublin

ABSTRACT

Our research aimed to evaluate the oxidant-antioxidant balance in rats exposed to the Ukrain and AMI drugs. The effect of 14-day administration of these drugs on selected parameters of oxidative stress in blood of rats has been investigated. The activity of antioxidant enzymes, namely glutathione peroxidase (GPX) and reductase (GR), and Total Antioxidant Status (TAS) were determined. It was noted that combined 14-day use of Ukrain (14 or 28 mg/kg) and AMI (10 mg/kg) significantly increased TAS and did not affect the activity of GPX and GR in comparison to groups receiving only Ukrain or AMI alone. While Ukrain administered to rats for 14 days in the above doses did not affect either the activity of the enzymes assayed or the level of TAS. After 2-week treatment with AMI at a dose of 10 mg/kg any statistically significant change of the enzymes activitiy or TAS concentration was not reported either, compared with the control group. The results indicate the beneficial effect of the combination of Ukrain and AMI drugs on the oxidant-antioxidant balance and increasing the antioxidant status in rats.

Keywords: Ukrain, amitriptyline, oxidative stress, rats

INTRODUCTION

The poisoning with antidepressants occurs quite frequently and constitutes an important cause of hospitalization in toxicology wards [2, 6, 8]. Particularly suicidal poisoning often involves ingestion of antidepressants. Fatal toxicity and case fatality indices provide results showing the greatest toxicity of tricyclic antidepressants (TCAs) in comparison with serotonin and noradrenaline reuptake inhibitors (SNRIs), selective serotonin reuptake inhibitors (SSRIs) and noradrenergic and specific serotonergic antidepressants (NaSSAs) [8]. Various xenobiotics intoxications lead to redox imbalance of the body. Oxidative-antioxidative system in conditions of homeostasis is the organism's defense mechanism against the harmful effects of reactive oxygen species (ROS). From the toxic effects of free radicals, human cells are protected by two antioxidant systems - enzymatic (superoxide dismutase, catalase, glutathione peroxidase and reductase) and non-enzymatic (glutathione, vitamin C, melatonin) [1, 17]. The imbalance between ROS generation and antioxidant capacities of the system causes an increase in oxidative processes and greater amount of free oxygen radicals. Thereby intensified oxidative stress can damage DNA, the structure and function of cells, tissues and organs [1, 2]. The recent research showed the abovementioned imbalance in patients hospitalized due to poisoning with antidepressants in the Regional Center of Clinical Toxicology. It was shown that intoxication with these drugs (amitriptyline mainly, the most widely used tricyclic antidepressant) caused a decrease of glutathione reductase activity and Total Antioxidant Status in blood samples of the patients. The results suggest an increase of oxidative processes, and thus an enhanced amount of free radicals and consequently intensified oxidative stress [2]. While our experiments revealed antioxidant activity of Ukrain drug in rats subjected to acute poisoning with ethylene glycol [10]. Ukrain is a semi-synthetic cytostatic (thiophosphoric acid derivative of *Chelidonium majus* L. alkaloids) which limits adverse effects of earlier administered cytostatics and also prolongs and improves quality of patients' lives [3, 9, 14, 16].

On the other hand, our previous studies conducted in rats and mice treated simultaneously with Ukrain and amitriptyline indicated the possibility of liver dysfunction and adverse effect on the kidneys [4, 5]. The results showed that Ukrain did not protect against adverse effect

Corresponding author
* Department of Toxicology, Medical University of Lublin,
8 Chodzki Str., 20-093 Lublin, Poland
e-mail: monika.grzywacz@umlub.pl

of amitriptyline and even suggested the possibility of hepatic and renal disorders when the above-mentioned drugs are administered together for a long time. In view of these findings, there is a question to ask whether adverse changes in the parameters of liver and kidney function are not associated with redox imbalance of the body.

Individual scientific reports on the influence of Ukrain and amitriptyline on the balance of redox system [2, 10] and the lack of clinical data about the impact of combined use of both drugs on this balance inspired this work. The aim of this study was to assess some parameters of oxidative stress in blood of rats pretreated with Ukrain and amitriptyline for 14 days. The activity of glutathione peroxidase (GPX) and reductase (GR) and Total Antioxidant Status (TAS) were determined.

MATERIAL AND METHODS

Drugs and chemicals. The following substances were used in the study: Ukrain (aqueous high-purity concentrate 1:30, Ukrainian Anti-Cancer Institute, Vienna, Austria), amitriptyline hydrochloride (AMI) from Sigma-Aldrich GmbH (Germany) and also *aqua pro injectione* (Polfa Lublin, Poland). Ready-made diagnostic kits (RANDOX Laboratories Ltd., United Kingdom) were used to determine: glutathione peroxidase (GPX) and reductase (GR) activities and Total Antioxidant Status (TAS).

Animals. The study was carried out in male Wistar rats (200-250 g) coming from licensed breeder. The animals were kept at room temperature (20±1°C) under a natural day-night cycle in constant environmental conditions (humidity, noise). They had access to food and water *ad libitum*. The experiments were approved by the Local Ethics Committee on Animal Experimentation of the Medical University of Lublin.

Treatments. Aqueous solutions of AMI (10 mg/kg) and Ukrain (14 or 28 mg/kg) were prepared *ex tempore* and administered intraperitoneally (ip) once daily for 14 days separated or combined in constant volume 0.5 ml/100 g of body weight. Our previous research [4, 5] proved the above-mentioned doses of AMI and Ukrain were effective. The control groups received the same amounts of *aqua pro injectione*.

Experimental protocols. The experimental groups consisted of eight animals each. Twenty-four hours after the last injection, the animals were decapitated and the blood was taken and divided, one part to heparin tubes (whole blood) and the second one to clot. The whole heparinised blood was used to estimate glutathione peroxidase activity (GPX). The second part of blood, collected to clot, was centrifuged for 10 minutes at 3000 rpm. The serum was taken to determine glutathione reductase activity (GR) and Total Antioxidant Status (TAS).

Statistical analysis. Results are expressed as mean ± SEM. Statistical significance among groups was determined by ANOVA test and p-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The dysfunction of cell antioxidant system contributes to adverse changes in the whole body and the development of lifestyle diseases such as atherosclerosis, hypertension, type II diabetes and also cancer [2]. It is obvious that the concentration of reactive oxygen species inside cells results not only from an increase in production, but is also a consequence of impaired elimination of oxygen free radicals. Therapy of cancer requires the use of several different drugs, not just the basic, i.e., cytostatics, but also adjuvants to relieve pain or depression. The prevalence of psychiatric disorders (major depressive disorder mostly) in cancer patients is assessed between 25% and 60% and this calculation is probably underestimated, partly because many symptoms of depression closely mirror the effects of cancer [15]. Tricyclic antidepressants (TCAs), including amitriptyline, are often used as auxiliary agents in the treatment of chronic pain associated with cancer, but also to stabilize mood and improve the patient's overall mental state [11, 12, 15]. Polypharmacotherapy of cancer is therefore a necessity, but can lead to a dangerous phenomenon of polypharmacy. The mechanism of tissue and organ damage as a result of such adverse drug interactions, may be based on the excessive generation of oxygen free radicals, antioxidant system disorders and finally oxidative stress intensity. Whereas in scientific literature there are few reports examined the impact of Ukrain or amitriptyline on the oxidant-antioxidant balance of the body [2, 10].

The oxidative stress parameters were assessed in rats pretreated with Ukrain and AMI for 2 weeks. The activity of antioxidant enzymes such as glutathione peroxidase (GPX) and glutathione reductase (GR) and Total Antioxidant Status (TAS) were determined in the blood. Glutathione peroxidase is an enzyme found in many tissues, but mainly in the blood and liver, and its main task is to protect cells against oxidative stress, especially against hydrogen peroxide. The final product of the reaction catalyzed by this enzyme is glutathione disulfide. This molecule is harmful to the cells because of its ability to inactivate proteins. It is removed through close cooperation of glutathione peroxidase and glutathione reductase, which is able to reproduce the reduced form of glutathione [17].

Our studies indicated that Ukrain (14 and 28 mg/kg) administered for 14 days simultaneously with AMI (10 mg/kg) caused an increase of Total Antioxidant Status (TAS) compared to the groups of animals receiving only

one of these drugs (Fig. 1). However, based on the obtained results it was observed that the combined treatment with Ukrain (14 and 28 mg/kg) and AMI (10 mg/kg) had no significant effect on the activity of antioxidant enzymes (GR, GPX) (Fig. 2, 3). It was also found that Ukrain applied alone changed neither the activity of GPX and GR nor Total Antioxidant Status (Fig. 1-3). Fourteen-day administration of amitriptyline did not affect any assessed oxidative stress parameters either (Fig. 1-3). These results are not compatible but different from research of other authors who showed that amitriptyline caused significant decrease of the activity of glutathione reductase (GR) and also the concentration of TAS [2]. It should be noted that such changes have occurred after toxic doses of amitriptyline. We applied the dose that is frequently used for pharmacological and behavioral studies in rats and should not exhibit toxic effects [7, 13].

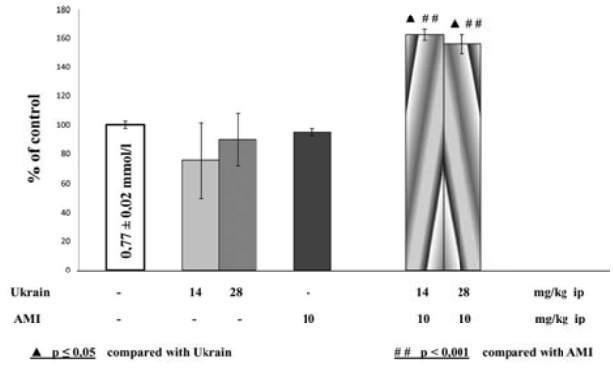

Fig. 1. Effect to 14-day treatment with Ukrain and AMI on Total Antioxidant Status

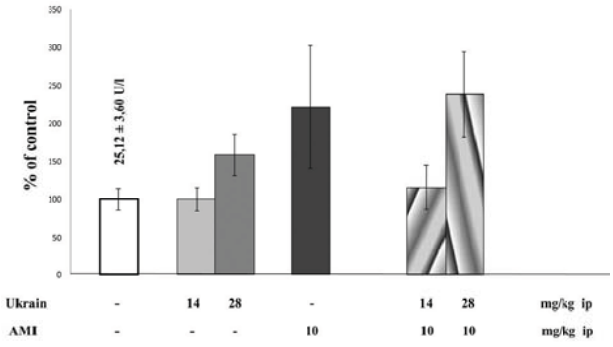

Fig. 2. Effect to 14-day treatment with Ukrain and AMI on glutathione reductase activity

The conducted experiments showed that 14-day simultaneous administration of Ukrain (14 or 28 mg/kg) and AMI (10 mg/kg) in rats appears to have beneficial effects on oxidant-antioxidant balance of the body. The abovementioned results appear to be surprising, since they do not correlate with previously observed and described, developing liver and kidney disorders [4, 5]. Perhaps the dysfunction of these organs is not advanced enough to

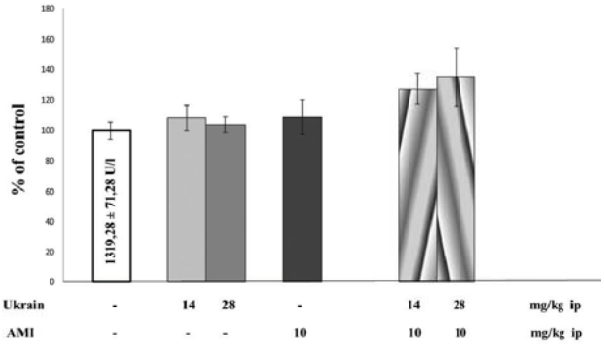

Fig. 3. Effect to 14-day treatment with Ukrain and AMI on glutathione peroxidase activity

cause changes in the balance of antioxidant system. Whereas, the inhibition of enzymes involved in glutathione pathway of reduction of superoxide forms, i.e., GPX and GR, and thus the weakening of the antioxidant system, are most frequently observed in lesions, including in the process of neurodegeneration and because of aging [17]. We observed the increase of Total Antioxidant Status in the blood of animals with no effect on the activity of glutathione peroxidase and reductase. Therefore, our results may indicate increasing amounts of antioxidants that protect every cell in the body from harmful changes caused by reactive oxygen species, such as the destruction of proteins, nucleic acids and lipids.

Acknowledgements: The authors wish to express their gratitude to dr J.W. Nowicky (Ukrainian Anti-Cancer Institute, Vienna, Austria) for the generous gift of Ukrain.

REFERENCES

1. Bilska A., Kryczyk., Włodek L.: Różne oblicza biologicznej roli glutationu (The different aspects of the biological role of glutathione). *Postępy Hig. Med. Dośw.*, 61, 438, 2007.
2. Drelich G. Lewandowska-Stanek H., Jagiełło-Wójtowicz E.: Zachwianie równowagi oksydoredukcyjnej w samobójczych zatruciach lekami przeciwdepresyjnymi (Imbalance of oxidoreductive status in suicidal antidepressant drugs poisoning). *Przegl. Lek.*, 64 (4-5), 258, 2007.
3. Gansauge F. et al.: The clinical efficacy of adjuvant systemic chemotherapy with gemcitabine and NSC-631570 in advanced pancreatic cancer. *Hepatogastroenterology*, 54 (75), 917, 2007.
4. Gawrońska-Grzywacz M. et al.: Influence of combined administration of Ukrain and tricyclic antidepressants on basic biochemical serum parameters of kidney function in rats. *Ann. UMCS Sect. DDD*, 24 (2), 17, 2011.
5. Gawrońska-Grzywacz M., Herbet M., Jagiełło-Wójtowicz E.: Effect of simultaneous treatment with Ukrain and amitriptyline on selected biochemical serum parameters in rodents. *Ann. UMCS Sect. DDD*, 23 (4), 129, 2010.
6. Gołacka E.: Zatrucia lekami z grupy TCA w materiale Regionalnego Ośrodka Toksykologii Klinicznej w Lublinie w roku 2002. *Przegl. Lek.*, 60, 289, 2003.
7. Haduch A., Wójcikowski J., Daniel W. A.: The effect of tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) and newer antidepressant drugs on the activity

and level of rat CYP3A. *Eur. Neuropsychopharmacol.*, 16, 178, 2006.
8. Hawton K. et al.: Toxicity of antidepressants: rates of suicide relative to prescribing and non-fatal overdose. *Br. J. Psychiatry*, 196 (5), 354, 2010.
9. Jagiełło-Wójtowicz E. et al.: Ukrain (NSC-631570) in experimental and clinical studies: a review. *Drugs Exptl. Clin. Res.,* 24 (5), 213, 1998.
10. Jagiełło-Wójtowicz E., Izdebska M.: Evaluation of antioxidative activity of Ukrain in alcohol-intoxicated rats. *Ann. UMCS Sect. DDD*, 20 (2), 175, 2007.
11. Kautio A. L. et al.: Amitriptyline in the prevention of chemotherapy-induced neuropathic symptoms. *Anticancer Res.*, 29 (7), 2601, 2009.
12. Kautio A. L. et al.: Amitriptyline in the treatment of chemotherapy-induced neuropathic symptoms. *J. Pain Symptom Manage.*, 35 (1), 31, 2008.
13. Kubera M., Basta-Kaim A., Górka Z.: The effect of amitriptyline on the cell-mediated immunity of stressed rats. *Pol. J. Pharmacol.*, 47, 137, 1995.
14. Nowicky J. W. et al.: Ukrain both as anticancer and immunoregulatory agent. *Drugs Exptl. Clin. Res.*, 18, 51, 1992.
15. Pasquini M. et al.: Detection and treatment of depressive and anxiety disorders among cancer patients: feasibility and preliminary findings from a liaison service in an oncology division. *Depress. Anxiety,* 23 (7), 441, 2006.
16. Uglyanitsa K. N. et al. : Ukrain: A novel antitumor drug. *Drugs Exptl. Clin. Res.*, 26 (5), 341, 2000.
17. Zabłocka A., Janusz M.: Dwa oblicza wolnych rodników tlenowych (The two faces of reactive oxygen species). *Postępy Hig. Med. Dośw.*, 62, 118, 2008.