XML-IS

Our reference: TB 1326

P-authorquery-vx

AUTHOR QUERY FORM

ELSEVIER	**Journal: TB** **Article Number: 1326**	**Please e-mail or fax your responses and any corrections to:** **E-mail: corrections.esch@elsevier.macipd.com** **Fax: +44 1392 285878**

Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list. Note: if you opt to annotate the file with software other than Adobe Reader then please also highlight the appropriate place in the PDF file. To ensure fast publication of your paper please return your corrections within 48 hours.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Click on the Q link to go to the location in the proof.

Location in article	Query / Remark: click on the Q link to go Please insert your reply or correction at the corresponding line in the proof
Q1	Please confirm that given names and surnames have been identified correctly.
Q2	Please check the address for the corresponding author that has been added here, and correct if necessary.
Q3	Please check the e-mail address, telephone/fax number of the corresponding author, and correct if necessary.
Q4	"Djordevic et al., 2010" has been changed to "Djordjevic et al., 2010" as per the reference list. Please confirm.
Q5	"Davidson et al., 2001" has been changed to "Davidson and Schiestl, 2001" as per the reference list. Please confirm.
Q6	"Williamson and Meister (1981)" has been changed to "Williamson and Meister (1991)" as per the reference list. Please confirm.
Q7	"Hadzy-Petrushev et al., 2011" has been changed to "Hadzi-Petrushev et al., 2011" as per the reference list. Please confirm.
Q8	"Gutierrez-Correa and Stopanni., 1997" has been changed to "Gutierrez-Correa and Stoppani., 1997" as per the reference list. Please confirm.
Q9	The resolution of Figure[s] 1-5 are too low to be used. Please provide better quality figure[s] of 300 dpi.

Thank you for your assistance.

Journal of Thermal Biology ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at SciVerse ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Highlights

L-2-oxothiazolidine-4-carboxylate influence on age- and heat exposure-dependent peroxidation in rat's liver and kidney

Nikola Hadzi-Petrushev [a], Nikola Jankulovski [b], Mishko Milev [a], Pavlina Filipovska [a], Hristo Gagov [c], Elizabeta Gjorgievska [d], Dine Mitrov [e], Ramadan Sopi [a], Kiril Hristov [c], Mitko Mladenov [a,c]

[a] Faculty of Natural Sciences and Mathematics, Institute of Biology, "Sts, Cyril and Methodius" University, P.O. Box 162, Skopje 1000, Macedonia
[b] Medical Faculty, "Sts, Cyril and Methodius" University, Skopje 1000, Macedonia
[c] Department of Membrane Ion Channels, Institute of Biophysics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[d] Faculty of Dental Medicine, "Sts, Cyril and Methodius" University, Skopje 1000, Macedonia
[e] Faculty of Veterinary Medicine, "Sts, Cyril and Methodius" University, Skopje 1000, Macedonia

► The L-2-oxothiazolidine-4-carboxylate impact on the combined stress model in rats was investigated. ► Young animals appeared to be more sensitive to the addition of the L-2-oxothiazolidine-4-carboxylate. ► L-2-oxothiazolidine-4-carboxylate had picky effect on cytosolic glutathione-utilizing or restore enzymes in rats' liver and kidney. ► L-2-oxothiazolidine-4-carboxylate effects are related to its ability to be converted to glutathione.

0306-4565/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtherbio.2011.12.013

Please cite this article as: Hadzi-Petrushev, N., et al., L-2-oxothiazolidine-4-carboxylate influence on age- and heat exposure-dependent peroxidation in rat's liver and kidney. J. Thermal Biol. (2012), doi:10.1016/j.jtherbio.2011.12.013

Journal of Thermal Biology ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at SciVerse ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

L-2-oxothiazolidine-4-carboxylate influence on age- and heat exposure-dependent peroxidation in rat's liver and kidney

Nikola Hadzi-Petrushev[a], Nikola Jankulovski[b], Mishko Milev[a], Pavlina Filipovska[a], Hristo Gagov[c], Elizabeta Gjorgievska[d], Dine Mitrov[e], Ramadan Sopi[a], Kiril Hristov[c], Mitko Mladenov[a,c,*]

[a] Faculty of Natural Sciences and Mathematics, Institute of Biology, "Sts, Cyril and Methodius" University, P.O. Box 162, Skopje 1000, Macedonia
[b] Medical Faculty, "Sts, Cyril and Methodius" University, Skopje 1000, Macedonia
[c] Department of Membrane Ion Channels, Institute of Biophysics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
[d] Faculty of Dental Medicine, "Sts, Cyril and Methodius" University, Skopje 1000, Macedonia
[e] Faculty of Veterinary Medicine, "Sts, Cyril and Methodius" University, Skopje 1000, Macedonia

ARTICLE INFO

Article history:
Received 10 June 2011
Accepted 27 December 2011

Keywords::
Antioxidant enzymes
Aging
Heat exposure
Wistar rats
Glutathione

ABSTRACT

To investigate the impact of acute heat exposure on maintenance of redox homeostasis and antioxidant balance related to aging, we have determined the GSH levels in the liver and kidney, and the activity of antioxidant enzymes in the same organs from Wistar rats at two different ages, 35 days and 18 months. The animals were housed individually in a special heated chamber maintaining a constant temperature of 40 ± 0.5 °C. The results showed that the level of endogenous GSH was significantly lower in aged than in young animals. In general, the activity of antioxidant enzymes in investigated tissues displayed an age-dependent decline. Indeed, we found unchanged CAT activity and decreased GPx activity with age. On the other hand acute heat exposure led to disproportion between peroxide metabolizing enzymes (CAT, GPx) and GR, thus promoting H_2O_2 accumulation and prooxidative state in the liver of young animals. The results for the impact of L-2-oxothiazolidine-4-carboxylate in combined stress model suggested that in spite of restore levels of GSH, the restoration of oxido-reductive balance might have only been partial due to irreversible alterations in antioxidant enzymes set by acute heat exposure and aging. Interestingly, young animals appeared to be more sensitive to the supplementation of the L-2-oxothiazolidine-4-carboxylate, likely because of the more extensive increase of GSH observed in young L-2-oxothiazolidine-4-carboxylate treated animals.

1. Introduction

Recently, many studies explored the impact of aging on stress tolerance using physiologically relevant challenges such as environmental heating (Hall et al., 2000a, 2000b; Kregel et al., 1995). In this direction, Zhang et al. (2003) have shown that tissues injuries in aged animals are associated with the increased production of reactive oxygen species (ROS). The same authors suggest that in aged animals, a decline in the redox status along with increased ROS production can lead to the extensive hepatocellular oxidative damage. On the other hand ROS are kept at physiologically optimal levels by antioxidant defense systems, including the array of antioxidant enzymes: cellular and mitochondrial superoxide dismutase, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and a nonenzymatic antioxidant such as reduced glutathione (GSH). Superoxide dismutase converts superoxide anions to H_2O_2, which is then transformed to water by CAT or by GPx. Glutathione reductase (GR) plays an essential role in cell defense against reactive oxygen metabolites by sustaining the reduced status of glutathione, whose reducing power is also necessary for GPx activity (Djordjevic et al., 2010). Also, the peroxide-removing action of the GSH system represents one of the most important redox buffers within a cell because of its overwhelming abundance (Davidson and Schiestl, 2001). In this direction recently we have shown that administration of L-2-oxothiazolidine-4-carboxylate (OTC) protects against heat-induced GSH depletion in rats' plasma by increasing the hepatocellular pool of cysteine available for GSH synthesis (Hadzi-Petrushev et al., 2011). Reed (1990) has published that OTC as a cysteine precursor, raises cellular glutathione levels by providing a source of cellular cysteine, (the rate-limiting substrate for

Abbreviations: ROS, reactive oxygen species; OTC, L-2-oxothiazolidine-4-carboxylate; GSH, glutathione; GPx, glutathione peroxidase; GR, glutathione reductase; CAT, catalase; H_2O_2, hydrogen peroxide; T_{co}, colorectal temperature; GSSG, glutathione disulfide; $HO^{\bullet}$, hydroxyl radical

* Corresponding author at: Faculty of Natural Sciences and Mathematics, Institute of Biology, "Sts, Cyril and Methodius" University, P.O. Box 162, Skopje 1000, Macedonia. Tel.: +389 2 3249 605; fax: +389 2 3228 141.
E-mail address: mitkom@pmf.ukim.mk (M. Mladenov).

0306-4565/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jtherbio.2011.12.013

glutathione biosynthesis). OTC is stable in plasma and is readily transported into the cells, where it is converted into cysteine by 5-oxo-L-prolinase (Meister, 1983). Moreover, Reed (1990) reported that OTC under in vitro conditions, raises cellular glutathione and reduces cellular damage induced by free radicals generated by ionizing radiation or other sources. OTC has also been shown to be effective as a nontoxic cysteine delivery system for glutathione synthesis in neonatal pigs and rats on a cysteine free diet (Aw et al., 1984; Ghibelli et al., 1995). These evidences indicate that increase of glutathione levels by supplementation of the exogenous OTC can be useful mean in the protection against oxidative injury in aged rats affected by heat exposure.

Our experiments were focused on the liver because it shows age-dependent evidence of increased cellular ROS production (Yen et al., 1994) and is a prime target of tissue injury with environmental challenges such as heat stress (Hall et al., 2000b; Kew et al., 1971). The kidneys were chosen because there is no previous study for kidney affecting acute heat exposure, aging, and OTC prevention. In this direction, on the basis of studies from Goad et al. (1987), Chawla et al. (1984) and Williamson and Meister (1991), another question was addressed as a result in the "difference in uptake" of OTC between hepatocytes and renal cells. Actually, the authors mentioned above stated that this difference in OTC uptake affects GSH synthesis in different extents at both tissues, in dependence of physiological conditions. From this reason we hypothesized that affected GSH synthesis and efflux in these conditions will counterbalance antioxidant enzymes activities as well. Also, this work represents a logical extension of our previous work (Hadzi-Petrushev et al., 2011), which was focused on age- and heat exposure-dependent redox changes in rat's blood plasma affected by L-2-oxothiazolidine-4-carboxylate.

2. Materials and methods

2.1. In vivo treatment and exposure

All experimental procedures were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Macedonian Center for Bioethics. The investigation was done conforming to the (or in compliance with the) Guide for the Care and Use of Laboratory Animals published by the Institutional Animal Care and Use Committee, April 1997, Oakland University, MI, USA. Anesthetics were applied according to the standards given by the guide of the Oakland University. Male Wistar rats ($n=80$) were used for all protocols and were maintained on a 12:12 light:dark cycle and fed standard rat chow and water ad libitum. All animals were divided into eight groups. Four groups consisted of 35 day old rats. They were further divided depending on treatment with OTC and heat exposure into young saline treated heat-unexposed (YSHU), young saline treated heat-exposed (YSHE), young OTC treated heat-unexposed (YTHU) and young OTC treated heat-exposed (YTHE) rats. The other four groups included 18 months aged rats, they were divided in a similar manner into aged saline treated heat-unexposed (ASHU), aged saline treated heat-exposed (ASHE), aged OTC treated heat-unexposed (ATHU) and aged OTC treated heat-exposed (ATHE) animals. Treated rats were injected intraperitoneally with OTC (6.5 mmol/kg body mass) on the day of sacrifice, 5 h prior to heat exposure. OTC was dissolved in physiological salt solution with pH=7.0. During the treatment period all animals were housed at 20 ± 2 °C.

The heat exposed rats were housed individually in a special heated chamber maintaining a constant temperature of 40 ± 0.5 °C and relative air humidity of 30–40%. During heat exposure, colorectal (co-re) temperature (T_{co}) was read by an electrical thermometer (Ellab TE 3) every 10 min until it reached the temperature of the chamber, and then the readings were taken at 2-min intervals. During the period of heating, the access to food and water was restricted. The exposure was terminated when T_{co} reached 42 ± 0.5 °C. The animals that did not survive the exposure term of 2 h were excluded from the study.

2.2. Preparation of samples

Animals were sacrificed by ether narcosis, and the abdomen was immediately incised. For the analyses, liver and kidney tissues were cut into pieces, cleaned off blood with external washing, frozen in liquid nitrogen and kept at −70 °C for further analysis. Briefly, both tissues of each animal were processed with manual maceration. The samples were sonicated by an ultrasonic homogenizer (Cole-Parmer Instrument—4710) in phosphate buffer (pH 7.4) and centrifuged for 10 min at 3500*g*. The supernatant was transferred to a fresh tube and a second centrifugation was performed for 10 min at 15000*g*. The supernatant from the second centrifugation was used for all assays. All analyses were performed at temperatures 0–4 °C.

2.3. Assays for enzymes activity

The levels of GSH in liver and kidney were determined by test reagents manufactured by Sigma: Glutathione Assay Kits. The measurement of GSH uses a kinetic assay in which catalytic amounts (nmoles) of GSH cause a continuous reduction of 5, 5′-dithiobis 2-nitrobenzoic acid (DTNB) to 5-thio-2-nitrobenzoic acid (TNB) and the GSSG formed is recycled by glutathione reductase and β-Nicotinamide Adenine Dinucleotide Phosphate-Reduced (NADPH).

GPx activity was determined by test reagents manufactured by Sigma: Glutathione Peroxidase Cellular Activity Assay Kits. Briefly, the kit uses an indirect determination method based on the oxidation of the reduced form of glutathione (GSH) to oxidized form of glutathione (GSSG) catalyzed by GPx, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase (GR) and NADPH (β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced).

The GR activity in samples of tissues was determined using the assay based on the reduction of glutathione (GSSG) by NADPH in the presence of glutathione reductase: Glutathione Reductase Assay Kits.

The CAT activities in tissues samples were determined using the Bio Assay Systems [Enzy Chrom Catalase Assay Kit (ECAT-100)] improved assay for direct measurement of catalase degradation of H_2O_2. The change in color intensity at 570 nm is directly proportional to the catalase activity in the samples.

Analyses for GSH, GPx, GR and CAT were carried out on the ELISA reader (Bio-Rad), while analyses for tissue protein levels were carried out on the spectrophotometer *Cintra 6*. The results are expressed as μg/mg protein for GSH and U/mg protein for GPx, GR and CAT. The tissue protein levels were determined using the method described by Lowry et al. (1951). All assays were independently performed in duplicate.

2.4. Statistical analysis

Data are expressed as means and standard errors of the means (SEM) and were analyzed using a one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test. The level of significance established for the test was $p<0.05$. SPSS version 14.0 was used.

Please cite this article as: Hadzi-Petrushev, N., et al., L-2-oxothiazolidine-4-carboxylate influence on age- and heat exposure-dependent peroxidation in rat's liver and kidney. J. Thermal Biol. (2012), doi:10.1016/j.jtherbio.2011.12.013

2.5. Chemicals

L-2-oxothiazolidine-4-carboxylate, NaCl, KCl, Na_2HPO_4, KH_2PO_4, $MgCl_2$, hexaamminecobalt (III) chloride, tris base, sodium citrate, $NaNO_2$, sulfanilamide, KOH, HCl, perchloric acid, guanidine hydrochloride, 1-chloro-2,4,-dinitrobenzene, glycine, NADPH, $NADP^+$, reduced glutathione, BSA, glutathione reductase from *Saccharomyces cerevisiae*, tert-butyl hydroperoxide, hydrogen peroxide, EDTA, methanol, ethanol, ethyl acetate, 5,5-dithiobis (2-nitrobenzoic acid), N-ethylmaleimide, 2,4-dinitrophenylhydrazine, trichloroacetic acid, 1,1,3,3-tetraethoxypropane and EDTA were obtained from Sigma–Aldrich.

3. Results

3.1. Time course for colorectal temperature changes during the experimental period

In (Fig. 1)-*inset*-A are shown phases in co-re temperature dynamics in all heat-exposed groups and control saline treated heat unexposed groups. During acute heat exposure, three phases in dynamics of co-re temperature were detected. The initial one was referred as primary hyperthermy, the second as temperature plateau and the third one as secondary hyperthermy (Mladenov et al., 2006). Primary hyperthermy ended somewhere about 30 min of heat exposure. As a result of limited time of exposure our results do not show clear difference between second and third phase. OTC treatment 30 min after heat exposure significantly prevented colorectal temperature increase in aged animals ($p<0.05$) in comparison with young animals. This is probably in relation with age-dependent development of different mechanisms for coping with acute heat exposure. On the other hand our results revealed that after 120 min of heat exposure, OTC treatment did not cause significant ($p=0.098$ and 0.079) changes in the duration of the second phase in both young and aged animal groups, respectively, when compared with saline treated animals (Fig. 1). During the experiment, two adult and one young untreated rat did not survive the heat exposure. The rate of survival depending on treatment with OTC did not differ much from that for untreated (one adult and two young treated animals died during the heat exposure)–data are not shown.

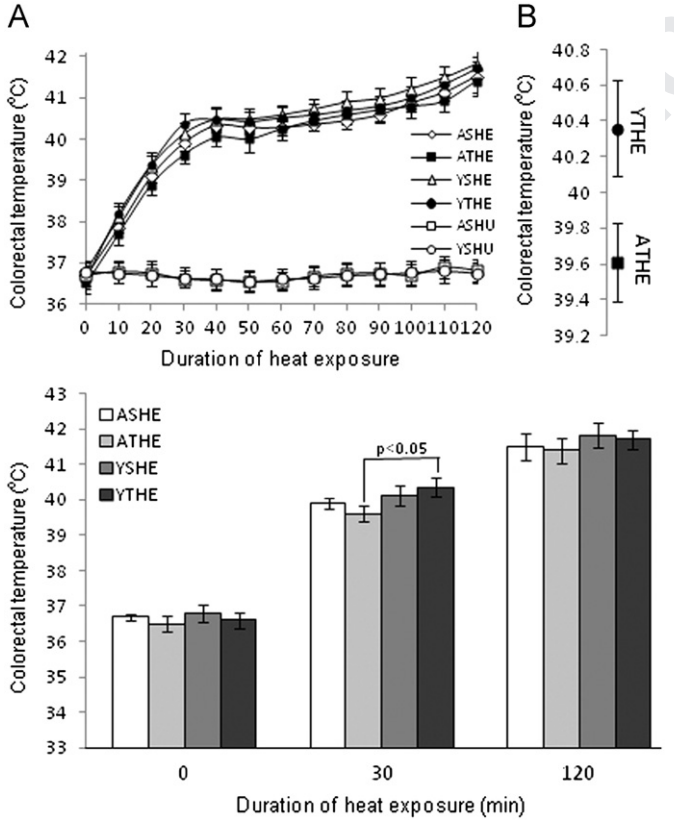

Fig. 1. Colorectal temperatures in heat exposed groups: before heat exposure and 30 and 120 min after heat exposure. *Inset-A*—Time course for colorectal temperature (T co-re) changes during the experimental period. *I* and *II* phases in dynamics of co-re temperature. *Inset-B*—magnification of statistical significance between YTHE and ATHE. YSHU—young saline treated heat-unexposed; YSHE—young saline treated heat-exposed; YTHU—young OTC treated heat-unexposed; YTHE—young OTC treated heat-exposed; ASHU—adult saline treated heat-unexposed; ASHE—adult saline treated heat-exposed; ATHU—adult OTC treated heat-unexposed; ATHE—adult OTC treated heat-exposed rats.

3.2. Influence of aging, OTC treatment and acute heat exposure on the GSH levels

The levels of GSH significantly ($p<0.001$) declined with aging in both the liver and kidney. Treatment with OTC reversed the effect of age in both young tissues ($p<0.001$, respectively), but not in aged kidney ($p=0.078$). Acute heat exposure caused a significant decrease of GSH levels in the kidneys of both young and aged rats ($p<0.05$, respectively). Comparisons between SHE versus THE animals at young or aged groups, respectively, shown that OTC treatment significantly ($p<0.05$) prevented diminution of GSH only in kidney of aged heat-exposed animals (Fig. 2). Interestingly, preventive effect of OTC treatment was not pronounced in YTHE kidney, where exposure caused GSH declination was not prevented as a consequence of treatment.

3.3. Influence of aging, OTC treatment and acute heat exposure on the GPx activity

Glutathione peroxidase (GPx), was found to be decreased significantly ($p<0.001$) with aging in both the liver and kidney. The statistical analyses revealed that after heat exposure the GPx activity significantly ($p<0.05$) was increased in liver of young animals, but there was no significant change in GPx activity in the kidney of young rats as well as in liver and kidney of aged rats. The treatment with OTC by itself or in function of aging or heat exposure did not cause significant changes in GPx activity neither in liver ($p=1.000$ for both young and aged rats) nor in kidney ($p=0.798$ and $p=1.000$ for young and aged rats) (Fig. 3).

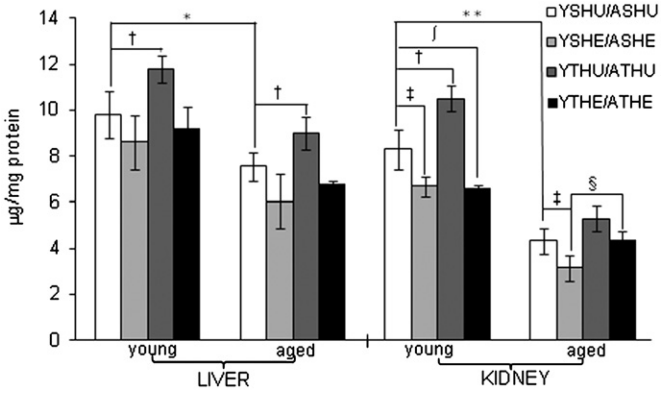

Fig. 2. Glutathione (GSH) levels in liver and kidney of the young and aged rats. Data are presented as mean ± SEM. *Effect of aging, †effect of OTC treatment, ‡effect of heat-exposure, §effect of OTC treatment in heat-exposed rats, ∫effect of OTC treatment and heat exposure. *,†,‡,§,∫; $p<0.05$; **; $p<0.001$. Abbreviations for groups are the same as in Fig. 1.

Please cite this article as: Hadzi-Petrushev, N., et al., L-2-oxothiazolidine-4-carboxylate influence on age- and heat exposure-dependent peroxidation in rat's liver and kidney. J. Thermal Biol. (2012), doi:10.1016/j.jtherbio.2011.12.013

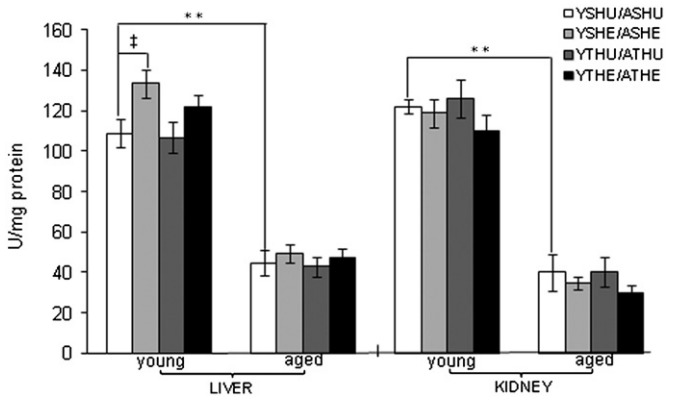

Fig. 3. Glutathione peroxidase (GPx) activity in liver and kidney. Data are presented as mean ± SEM. *Effect of aging, ‡effect of heat-exposure. ‡$p < 0.05$; **$p < 0.001$. Abbreviations for groups are the same as in Fig. 1.

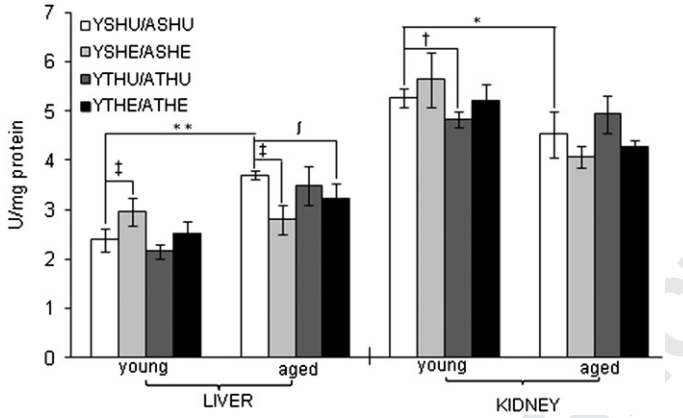

Fig. 4. Glutathione reductase (GR) activity in liver and kidney. Data are presented as mean ± SEM. *Effect of aging, ‡effect of heat-exposure, ∫effect of OTC treatment and heat exposure. ∗,‡,∫; $p < 0.05$; ∗; $p < 0.001$. Abbreviations for groups are the same as in Fig. 1.

3.4. Influence of aging, OTC treatment and acute heat exposure on the GR activity

The data presented in Fig. 4, clearly demonstrate that the average of GR activity in the liver significantly ($p < 0.001$) increased with aging, whereas in the kidney the GR activity significantly ($p < 0.05$) decreased with increase of age. Acute heat exposure also caused a significant increase of GR activity in the liver of young animals ($p < 0.05$), while the effect of heat exposure on the GR activity in the liver of aged animals was opposite and it caused a significant ($p < 0.001$) decrease in GR activity. There were no significant changes in GR activity in the kidney of both young and aged rats after acute heat exposure. The treatment with OTC by itself caused a significant ($p < 0.05$) decrease of GR activity in kidney of young animals. The preventive effect of OTC treatment was not pronounced in ATHE liver, where treatment does not stop GR activity decrease as a consequence of exposure.

3.5. Influence of aging, OTC treatment and acute heat exposure on the CAT activity

There were no significant age-related differences of CAT activity in the liver and kidney ($p = 0.283$ and $p = 0.254$, respectively). The OTC treatment significantly ($p < 0.05$) increased CAT activity only in liver of young rats. Acute heat exposure affected the CAT activity only in aged rats and led to a substantial decrease of CAT

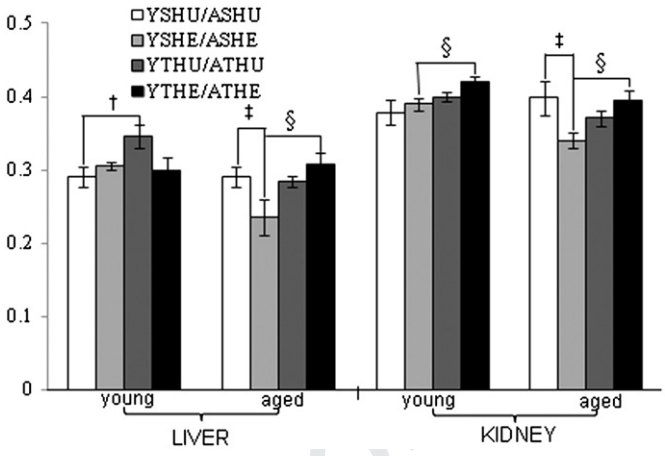

Fig. 5. Catalase (CAT) activity in liver and kidney. Data are presented as mean ± SEM. *Effect of aging, †effect of OTC treatment, ‡effect of heat-exposure, §effect of OTC treatment in heat-exposed rats. †,‡,§; $p < 0.05$. Abbreviations for groups are the same as in Fig. 1.

activities in both tissues of interest ($p < 0.05$, for both tissues). It is interesting that with exception of young livers, OTC treatment in function of heat exposure did not cause any significant changes in CAT activity (Fig. 5).

4. Discussion

In the present study we have investigated whether both aging and acute heat exposure of male Wistar rats alters antioxidant capacity in the liver and the kidney, with regard to antioxidant enzymes activity, and whether these alterations could be prevented by treatment with OTC. Under these conditions and having in mind that both organs have high oxygen consumption rates, we have addressed the idea that both investigated tissues may be susceptible to oxidative damage by free radicals generated during heat exposure (Mladenov et al., 2006, 2008). The rate of tissues damage probably can be defined as the rate of deficiency in antioxidant enzymes defense against ROS.

Because we found a decrease in GSH level in our study, it seems that targeted organs are highly susceptible to oxidative stress during aging. In this direction Morrison et al. (2005) showed that CAT activity significantly decreases with aging, while the activity of GPx remains unchanged during aging. This outcome was not observed in our study and we found that CAT activity does not change and the GPx activity decreases with aging of rats, in both the liver and kidney. Interestingly, with exception of the liver of young animals, we did not observe changes in GPx activity under acute heat exposure as well. The lack of alteration of the GPx activity in aged heat-exposed rats may also be a consequence of changes in glucocorticoid hormones (Jose et al., 1997), whose level is highly affected during acute stress (Michel et al., 2007). This assumption was further corroborated by our findings for down-regulating effect of heat exposure on the GR activity in the liver of adult heat-exposed animals, since the expression of this enzyme is known to be inhibited by increase of H_2O_2 (Gutierrez-Correa and Stoppani, 1997). GR is known to be an essential enzyme in regulation of overall homeostatic oxido-reductive balance in any living cell, and the lack of GR activity was previously demonstrated in numerous clinical pathologies (Loos et al., 1976). Therefore, we documented that acute heat exposure led to disproportion between peroxide metabolizing enzymes (CAT, GPx) and GR, thus promoting H_2O_2 accumulation and pro-oxidative state in the liver of young rats.

Please cite this article as: Hadzi-Petrushev, N., et al., L-2-oxothiazolidine-4-carboxylate influence on age- and heat exposure-dependent peroxidation in rat's liver and kidney. J. Thermal Biol. (2012), doi:10.1016/j.jtherbio.2011.12.013

In saline-treated control rats, heat exposure caused a decrease in CAT activity in both tissues, only in aged rats. On the other hand, Zuo et al. (2000), have shown that increased CAT activity potentiates heat stress-induced cell death via scavenging of H_2O_2. The same authors stated that increased CAT activity may not be observed immediately after heat stress to allow unimpeded HSF1 activation and subsequent development of thermo tolerance. Moreover, they assume that subtle changes in CAT activity cannot be excluded when these data are considered with the reduction in liver GSH. This assumption was supported by our finding of reduction in GSH level during heat exposure, in kidneys of both young and aged animals. Also, in this study, we have shown that reductions in hepatic GSH are counterbalanced by increases in hepatic GR activity in young rats. The last relationship indicates that maintaining a balance between enzymes and proteins responsible for each step of removal of ROS would seem to be a prerequisite for maintaining the redox homeostasis.

It may be of special interest to mention that GPx was not restored in spite of the prominent increase in the level of liver GR activity during aging. On the contrary, highly increased GR activity in the liver of young animals as a consequence of heat exposure was followed with increased GPx activity. Young animals had also an increase of the level of GSH in liver and kidney and an increase of the CAT activity only in liver as a consequence of treatment with OTC. In line with studies of Vergauwen et al. (2003), our study shows that treatment with OTC increases reliance on CAT activity after heat exposure to counterbalance the reduction in cellular GSH and keep H_2O_2 levels under control in young animals. These results represent verification for the ability of OTC to increase endogenous antioxidant capacity in liver of young animals. Indeed, almost in all tested parameters in heat exposed groups, there is a clear tendency of OTC treatment to prevent or even remedy heat induced alteration; however these changes are still not statistically significant with exception of the GSH levels.

Taken together, the results for OTC impact on combined stress model suggest that in spite of restored levels of GSH, the restoration of oxido-reductive balance might have only been partial due to irreversible alterations in antioxidant enzymes set by aging and acute heat exposure.

In summary, the fluctuations in antioxidant enzymes activity observed in this study suggest that aged animals are characterized by a lack of antioxidant enzymes activity. Interestingly, young animals appeared to be more sensitive to the addition of the OTC, likely because of the more extensive increase of GSH observed in young OTC treated animals.

Finally, we conclude that OTC had particular effect on cytosolic glutathione-utilizing or restoring enzymes in rats' liver and kidney, which indicated that its effects are related mainly to its ability to be converted to GSH. Although, OTC have been explored for use in treating different stress-related conditions, because of the complexity of the reactions that are involved, the area is far from being understood and further studies will be required.

Funding

The authors gratefully acknowledge the financial support from the *Ministry of Education and Research of Macedonia*. The Biological Institute in the Faculty of Natural Sciences at the University "St Cyril and Methodius" in Skopje also supported this research.

Acknowledgments

The authors wish to thank Prof. Dr. Anton Kossev from Institute of Biophysics, Bulgarian Academy of Sciences in Sofia for assistance with manuscript preparation. Also, the authors wish to thank Oliver Nikolovski and Olga Rusimovich for technical assistance.

References

Aw, Y.T., Oktens, M., Kaplowitz, N., 1984. Inhibition of glutathione efflux from isolated rat hepatocytes by methionine. J. Biol. Chem. 259, 9355–9358.

Chawla, R.K., Lewis, W.F., Kutner, H.M., Bate, M.D., Roy, G.R.B., Rudman, D., 1984. Plasma cysteine, cystine, and glutathione in cirrhosis. Gastroenterology 87, 770–776.

Davidson, J.F., Schiestl, R.H., 2001. Mitochondrial respiratory electron carriers are involved in oxidative stress during heat stress in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 21, 8483–8489.

Djordjevic, J., Djordjevic, A., Adzic, M., Niciforovic, A., Radojcic, A.B., 2010. Chronic stress differentially affects antioxidant enzymes and modifies the acute stress response in liver of Wistar rats. Physiol. Res. 51, 729–736.

Ghibelli, L., Coppola, S., Rotilio, G., Lafavia, E., Maresca, V., Ciriolo, M.R., 1995. Non-oxidative loss of glutathione in apoptosis via GSH extrusion. Biochem. Biophys. Res. Commun. 216, 313–320.

Goad, M.E., Tryka, F.A., Witschi, P.H., 1987. Acute respiratory failure induced by bleomycin and hyperoxia: pulmonary edema, cell kinetics, and morphology. Toxicol. Appl. Physiol. 90, 10–22.

Gutierrez-Correa, J., Stoppani, O.A., 1997. Inactivation of yeast glutathione reductase by Fenton systems: effect of metal chelators, cateholamines and thiol compounds. Free Radical Res. 27, 543–555.

Hadzi-Petrushev, N., Hristov, K., Jankulovski, N., Mladenov, M., 2011. L-2-oxothiazolidine-4-carboxylate influence on age- and heat exposure-dependent redox changes in rat's blood plasma. J. Physiol. Sci. 6, 437–442.

Hall, D.M., Buettner, G.R., Oberley, L.W., Hu, L., Matthes, R.D., Gisolfi, C.V., 2000a. Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. Am. J. Physiol. Heart Circ. Physiol. 280, 509–521.

Hall, D.M., Xu, L., Drake, V.J., Oberley, L.W., Oberley, T.D., Moseley, P.L., Kregel, K.C., 2000b. Aging reduces adaptive capacity and stress protein expression in the liver after heat stress. J. Appl. Physiol. 89, 749–759.

Jose, H.J., Berenice, S.G., Cecilia, V.R., 1997. Induction of antioxidant enzymes by dexamethasone in the adult rat lung. Life Sci. 60, 2059–2067.

Kew, M.C., Berson, I., Seftel, H.C., 1971. The diagnostic and prognostic significance of serum enzyme changes in heatstroke. Trans. R. Soc. Trop. Med. Hyg. 65, 325–330.

Kregel, K.C., Moseley, P.L., Skidmore, R., Gutierrez, J.A., Guerriero, V., 1995. HSP70 accumulation in tissues of heat-stressed rats is blunted with advancing age. J. Appl. Physiol. 79, 1673–1678.

Loos, H., Roos, D., Weening, R., Houwerzijl, J., 1976. Familial deficiency of glutathione reductase in human blood cells. Blood 48, 53–62.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Meister, A., 1983. Selective modification of glutathione metabolism. Science 220, 472–477.

Michel, V., Peinnequin, A., Alonso, A., Buguet, A., Cespuglio, R., Canini, F., 2007. Effect of glucocorticoid depletion on heat-induced Hsp70, IL-1beta and TNF-alpha gene expression. Brain Res. 1164, 63–71.

Mladenov, M., Gjorgoski, I., Stafilov, T., Duridanova, D., 2006. Effect of vitamin C on lipid hydroperoxides and carbonyl groups content of rat plasma depending on age and acute heat exposure. J. Therm. Biol. 31, 588–593.

Mladenov, M., Tanska, V., Vitkovska, T., Gjorgoski, I., Dinevska-Kofkarovska, S., Stafilov, T., Hristov, K., Duridanova, D., 2008. Evidence for the influence of vitamin C on age- and heat exposure-dependent deterioration of biochemical function in rat's liver and kidney. J. Therm. Biol. 33, 431–436.

Morrison, J.P., Coleman, M.C., Aunan, E.S., Walsh, S.A., Spitz, D.R., Kregel, K.C., 2005. Aging reduces responsiveness to BSO- and heat stress induced perturbations of glutathione and antioxidant enzymes. Am. J. Physiol. Regul. Integr. Comp. Physiol. 289, 1035–1041.

Reed, D.J., 1990. Glutathione: toxicological implication. Annu. Rev. Pharmacol. Toxicol. 30, 603–631.

Vergauwen, B., Pauwels, F., Beeumen, J.J., 2003. Glutathione and catalase provide overlapping defenses for protection against respiration-generated hydrogen peroxide in *Haemophilus influenzae*. J. Bacteriol. 185, 5555–5562.

Williamson, J.M., Meister, A., 1991. Stimulation of hepatic glutathione formation by administration of L-2-oxothiazolidine-4-carboxylate, a 5-oxo-L-prolinase substrate. Proc. Natl. Acad. Sci. 78, 936–939.

Yen, T.C., King, K.L., Lee, H.C., Yen, S.H., Wei, Y.H., 1994. Age-dependent increase of mitochondrial DNA deletions together with lipid peroxides and superoxide dismutase. Free Radical Biol. Med. 16, 207–214.

Zhang, H.J., Xu, L., Drake, V.J., Xie, L., Oberley, L.W., Kregel, K.C., 2003. Heat-induced liver injury in adult rats is associated with exaggerated oxidative stress and altered transcription factor activation. FASEB J. 17, 2293–2295.

Zuo, L., Christofi, F.L., Wright, V.P., Liu, C.Y., Merola, A.J., Berliner, L.J., Clanton, T.L., 2000. Intra- and extracellular measurement of reactive oxygen species produced during heat stress in diaphragm muscle. Am. J. Physiol. Cell Physiol. 279, 1058–1066.

Please cite this article as: Hadzi-Petrushev, N., et al., L-2-oxothiazolidine-4-carboxylate influence on age- and heat exposure-dependent peroxidation in rat's liver and kidney. J. Thermal Biol. (2012), doi:10.1016/j.jtherbio.2011.12.013