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SHORT COMMUNICATION

Determination of Glutathione Levels and Turnover Rates in the Brain and Liver of Mice Treated with Cadmium

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Abstract

Cadmium is a toxic metal that affects many organ systems in the body. Medetomidine is an alpha-2 adrenoceptor agonist reported to reduce glutathione (GSH) levels in tissues. We used the effect of medetomidine to determine GSH levels and turnover rates in the brain and liver of mice acutely treated with cadmium. Female mice were treated with either saline (control) or cadmium chloride at 2 mg cadmium/5 ml saline/kg of body weight, itraperitoneally (ip), followed one hour later with medetomidine at 0.2 mg/kg of body weight, ip. Five hours after the medetomidine administration, the mice were sacrificed using terminal ether anesthesia to obtain the whole brain and liver. GSH level was determined in the homogenized brain or liver spectrophotometrically. Turnover parameters (efflux rate constant-k, turnover time, and turnover rate) of GSH were estimated by a steady state kinetic relationship. The levels of GSH after medetomidine or cadmium + medetomidine treatments were reduced in the brain (12.4% and 11.4%, respectively) and liver (3.8% and 15.1%, respectively) of mice in comparison with respective control values. Cadmium reduced GSH trunover rate in the brain of mice by 8% with a corresponding decrease in k value by 8% and an increase of 9% in the turnover time. In the liver, it increased the turnover rate by 320% with a corresponding increase in k value by 319% and a reduction of turnover time by 76%. In conclusion, cadmium differentially affected GSH levels and turnover rates in the brain and liver of mice. Medetomidine administration was found to be a potential simple tool to determine GSH turnover and related parameters in tissues.

Keywords: alpha-2 agonist, cadmium, glutathione, medetomidine, turnover rate.

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INTRODUCTION

Cadmium (Cd) is a multi target toxic metal, which adversely affects many organ systems in the body [1]. It induces changes in glutathione (GSH) levels and homeostasis in several organs [2,3]. Acute Cd intoxication in rats and mice has been reported to reduce GSH level in various tissues in the body [2-4]. Medetomidine is an alpha-2 adrenoceptor agonsit used as a sedative in animals [5]. Reports have indicated that medetomidine and other alpha-2 agonists modulate GSH metabolism with a rdeuction in its levels in the liver and brain of experimental animals [6,7].

Glutathione, which is a tripeptide (cysteine, glycine, and glutamic acid) exists intracellulary as reduced (the most abundant) or oxidized forms [8]. It mainly protects many organ systems such as the liver and the brain from oxidative reactive species preventing oxidative stress [8,9]. Another crucial function of GSH is to detoxify many toxicants such as metals [10] and drugs [11] and its depletion plays an important role in apotosis mechanisms in cancer therapy [12]. Furthermore, GSH plays an essential role as a cofactor for antioxidant and detoxification enzymes [8,9]. The turnover rate parameters of GSH, which undergoes depletion and synthetsis in the cellular pools, are important in disease conditions such hepatitis and neurodegenerative disorders [8,12]. In the present report, an attempted was made to use the unique property of medetomidine, which is the reduction of tissue GSH level, for the first time to determine GSH turnover rates in the brain and liver of mice acutely treated with Cd.

MATERIALS & METHODS

Adult female Swiss mice (body weight 20-30 g) were housed under satndard conditions of temperature with laboartory feed and water *ad libitum*. The Committee of Postgraduate Studies at the College of Veterinary Medicine, University of Mosul, Iraq has approved the present study according to the institutional regulations and ethics on the animal use and handling in biomedical research in compliance with ARRIVE (Animal Research: Reporting of *In Vivo* Experiments; https://www.nc3rs.org.uk/arrive-guidelines) guidelines and the Guide for the Care and Use of Laboratory Animals (https://www.ncbi.nlm.nih.gov/books/NBK54050/). The mice were not fasted before treatments, and food was withheld from them during the experiment with free access to water.

This is a case-control study in which mice (4-6/group) were treated with either a single dose of saline (5 ml/kg-

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control group) or Cd chloride ($CdCl_2.2½H_2O$, BDH, Poole, UK) at 2 mg Cd/5 ml saline/kg of body weight, itraperitoneally (ip), followed one hour later with medetomidine (Domitor 1 mg/ml injectable solution, Orion Corp. Farmos, Turku, Finland) at 0.2 mg/kg of body weight, ip. The doses of medetomidine and Cd were chosen from literature [2,13-15]. Five hours after the medetomidine administration, the mice were sacrificed using terminal ether anesthesia [16] to obtain the whole brain and liver. Homogenizing tubes containing tissues were immersed in crushed ice, and tissues were homogenized in 6% trichloroacetic acid solution with an electric homogenizer at 400 rpm for 30 seconds. After centrifuging the homogenate at 3000 rpm for 20 min, the supernant was used to determine the concentration of GSH using a spectrophotometric method [17].

We applied a steady state kinetic relationship on the rate of decline of brain or liver GSH (0 vs 5 hour) to estimate turnover parameters of GSH (efflux rate constant, turnover time, and turnover rate) [18,19]. Using the online program Omni Calculator (Chemistry Calculators, https://www.omnicalculator.com/chemistry), we calculated the decay half life, efflux rate constant, turnover time, and turnover rate of GSH under the influence of Cd in comparison to saline-control using the following equations reported earlier [18,19]:

$\text{Log [GSH]} = \text{Log [GSH]}_0 - 0.434kt$

$\text{Slope} = 0.434k$

$-k = \text{slope}/0.434$

$\text{Turnover time} = 1/k$

$\text{Turnover rate} = [\text{GSH}]_0 \times k$

where, $[\text{GSH}]_0$ is the steady state GSH level at time 0, [GSH] is the GSH level at t time (5 hours), k is the efflux rate constant. These calculations were also statistically verified by the linear regression using the statistical sofware program Past 4.11 (https://www.nhm.uio.no/english/research/resources/past/).

Tissue concentrations of GSH were subjected to analysis of variance followed by the least significant difference test at $p < 0.05$, using the statistical sofware program Past4.11. Percent changes of turn over rate parameters are included in data presentation.

RESULTS & DISCUSSION

Table 1 shows that the levels of GSH after medetomidine or Cd + medetomidine treatments were reduced in the brain (12.4% and 11.4%, respectively) and liver (3.8% and 15.1%, respectively) of mice in comparison with respective control values. The reductions in brain and liver GSH levels in the Cd + medetomidine group were statistically significant compared with the corresponding control values at $p < 0.05$ (Table 1). Using the changes in the levels of GSH (Table 1), we calculated GSH turnover parameters in the brain and liver as described above. Table 2 shows that Cd reduced GSH trunover rate in the brain of mice by 8% with a corresponding decrease in the k value by 8% and an increase of 9% in the turnover time. In the liver, Cd markedly increased the turnover rate by 320% with a corresponding increase in the k value by 319% and an a reduction of turnover time by 76%.

The present estimates of GSH turnover parameters in the brain and liver of Cd-treated mice suggest a differential toxic effects of Cd on the organs. These effects could be attributed to the differential distribution of Cd in the liver

Table 1. Glutathione level in mice treated with cadmium (2 mg/kg) and/or medetomidine (0.2 mg/kg) intraperitoneally

Treatment	Glutathione level (µmol/g tissue)	
	Brain	Liver
Saline-control	2.02 ± 0.21	7.29 ± 0.54
Saline + Medetomidine	1.77 ± 0.16	7.01 ± 0.49
Cadmium + Medetomidine	1.79 ± 0.09*	6.19 ± 0.60*

Values are mean ± SE of the mean of 4-6 mice/group.
*Significantly different from the corresponding control value, $p < 0.05$.
Medetomidine was injected one hour after the cadmium administration, and glutathione level was determined 5 hours later.

Table 2. The glutathione turnover in the brain and liver of mice treated with cadmium at 2 mg/kg, intraperitoneally

Parameters	Control		Cadmium	
	Brain	Liver	Brain	Liver
Half-life (h)	26.23	88.49	28.67	21.19
k (h^{-1})	0.0264	0.0078	0.0242	0.0327
Turnover time (h)	37.85	127.66	41.36	30.57
Turnover rate (µmol/g/h)	0.0533	0.0569	0.049	0.2384

Values were drived from the steady state equation $\text{Log [GSH]} = \text{Log [GSH]}_0 - 0.434kt$ [13,14] using the software Omni Calculator (Chemistry Calculators, https://www.omnicalculator.com/chemistry)

and the brain, attaining high concentration in the liver after the Cd adiministration [20,21] with subsequent differential effects on GSH levels, which is manifested as significant stimulation of GSH production in the liver [2,22,23]. In an experiment in mice, it was reported that Cd injection alone at 2 mg/kg, ip differentially increased GSH level in the brain (12%) and reduced it in the liver (5.5%) [15]. Kumar et al. [4] reported reduced hepatic GSH levels in rats treated with Cd. However, they did not report the turnover rate of GSH under the influence of Cd. Furthermore, liver is known to induce GSH production, hence the turnover rate, under stressful toxicant conditions, as is the case with Cd [24,25]. The present observation of high turnover rate of GSH in the liver could be attributed to such a stressful effect of Cd on the organ. Another contributing factor to different turnover responses between the liver and brain, is the inherently high GSH levels and cysteine pools in the liver compared to those of the brain, which might explain, in part, the low turnover rate of GSH in the brain [26,27]. Within this context, rapid metabolic rate of GSH was reported by several studies in the liver vs. the brain of rats or mice [26-28]. The rising level of tissue GSH within hours is associated with a long half life ranging between 2 to 29 hours and a high level of cysteine pool [29] with rapid turnover rate in the liver [30].

CONCLUSION

In conclusion, Cd differentially affected GSH levels and turnover rates in the brain and liver of mice. Treatment of mice with medetomidine was found to be a potential simple tool to determine GSH turnover and related parameters in tissues. Additional studies are needed to apply the present method on different pools of GSH in the organ systems as well as different parts of the brain in laboratory animals under stressful toxicant conditions.

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Authors' contributions

FKM concetulaized the study, designed the experiments, shared in calculations, interpreted results and drafted the manuscript. BKA executed the experiments, shared in calculations and drafting the manuscript.

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