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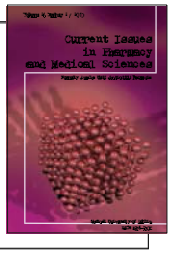

Effect of thyroxine on cardiac GLUT4 changes induced by doxorubicin

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ABSTRACT

Doxorubicin is an efficient anticancer drug that causes a dose-dependent cumulative cardiotoxicity as one of the most serious side effects. This cardiotoxicity may develop for months or years leading to heart failure that is not curable. It is generally believed that the mechanism of these phenomena is followed by periodical, progressive oxidative damage in mitochondria triggered by doxorubicin. Serious disturbance in mitochondria may activate glycolysis as an alternative pathway to ATP synthesis. The fuel for this process is glucose, which is transported into cells via GLUT4. The objective of this study was to test the thesis that thyroxine modulates changes in cardiac expression of GLUT4 in rats receiving doxorubicin. Rats were intraperitoneally treated with doxorubicin (1.5 mg/kg) once a week for ten weeks. Apart from doxorubicin, thyroxine was simultaneously given in drinking water (0.2 or 2.0 mg/l) for fourteen weeks. The study confirmed that doxorubicin increases cardiac concentration of mRNA and protein for GLUT4. Thyroxine had no significant effect on mRNA and protein of GLUT4 changes induced by doxorubicin.

Keywords: doxorubicin, throxine, GLUT4, heart

INTRODUCTION

It has long been known that doxorubicin and the upper level of thyroxine can disturb the physiological function of heart. Recent studies have established that the side effect of doxorubicin and thyroxine poses a similar cardiac target. Both agents lead to free radical generation in mitochondria [12, 17]. Interestingly, free radical overproduction in mitochondria can be caused by up and down regulation of electron transfer in mitochondrial chain regardless of the factor that caused it. On this basis, we assumed that cardiac side effects caused by the anticancer drug could be modified by thyroxine.

Doxorubicin in cardiomyocyte mitochondria is mainly reduced by NADPH-dependent flavin enzyme [5], which initiates reactions cascade leading to oxidative damage of mitochondrial DNA (mtDNA). The gene encoding this enzyme is under control of triiodothyronine (active metabolite of thyroxine). Iodothyronine hormones are key factors in the regulation of genes encoding enzymes responsible for producing NADPH [12]. NADPH is not only used as donor of electron for reducing doxorubicin but is also a crucial molecule in antioxidative defense [13]. Summing up, these mechanisms allow us to assume that thyroxine can exaggerate consequences of oxidative stress initiated by doxorubicin.

Reactive oxygen species cause DNA damages, the consequence of which is mutation of mitochondrial proteins [16] and disturbance in oxidative phosphorylation and as a result of diminishing of ATP synthesis. Inhibition of electron transfer via the respiration chain causes secondary free radical generation whereby even doxorubicin was eliminated from the organism [3]. It is assumed that this circle of oxidative damages repeats constantly and the clinical silent changes eventually become overt.

The main substrates for ATP synthesis in cardiomyocytes in physiological conditions are free fatty acids, lactate and -to a lesser extent- glucose [14]. Cardiac cells produce ATP mainly from these substrates in mitochondrial oxidative phosphorylation (98%), and in extremely low extent via glycolysis pathway (about 2%) [18]. The decreasing ATP synthesis caused by doxorubicin may

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trigger adaptive processes in cardiomyocytes. One of them is the compensatory increasing activity of glycolysis [15]. Since the fuel for glycolysis is glucose and not free fatty acids, a cell must transport it in a higher amount. The double lipid cell membrane is impermeable for simple carbohydrates, thus glucose must be transported into the cells [10]. In cardiomyocytes glucose is transported by Na^+ – independent GLUT transporter.

For these reasons, the objective of this study was to test the thesis that thyroxine modulates changes in cardiac expression of GLUT4. In addition, blood glucose level was assessed.

MATERIAL AND METHODS

The Local Bioethical Committee of the Medical University of Lublin approved the experimental protocol. The study was conducted on sexually mature albino rats of Wistar CRL: (WI) WUBR strain, obtained from a commercial breeder (Warsaw-Rembertow, Poland). The animals with the initial body weight of 160-195 g were maintained in stable conditions at 22°C with a 12 h light/dark cycle and were given standardized granulated fodder LSM (AGROPOL, Poland). All the animals were randomly divided into six groups which received respectively: saline (control); doxorubicin 1.5 mg/kg (DOX); thyroxin 2.0 mg/L and doxorubicin 1.5 mg/kg (2T4+DOX); - thyroxin 4.0 mg/L and doxorubicin 1.5 mg/kg (4T4+DOX); thyroxin 2.0 mg/L (2T4) and thyroxin 4.0 mg/L (4T4). Doxorubicin (Ebewe, Austria) was intraperitoneally injected (1.5 mg/kg) once a week for ten weeks. Thyroxine (Sigma, USA) was administered in drinking water in two doses. Thyroxine administration was started one week before doxorubicin injection and finished three weeks after completing doxorubicin treatment. The animals were anesthetized with pentobarbital. The blood and heart were collected during autopsy. The heart was washed with 20 mL of saline, and then sectioned along the interventricular and coronal groove. The left ventricular wall was placed in liquid nitrogen and stored at -75°C until the molecular analyses were conducted. After thawing, the tissue sections were homogenized in 20 mM phosphate buffer at pH 7.4 at a ratio of 0.5 g of tissue in 2 cm3of the buffer. A homogenizer with a Teflon piston (Glas-col, USA) was used for homogenization (5 min at 4000 rpm). The obtained homogenates were centrifuged for 20 minutes at 14 000 rpm at 4°C.

RESULTS

In the group receiving separately doxorubicin and thyroxin, significant increase in plasma glucose level was observed (Fig. 1). However, there was no important additive effect when both agents were given together.

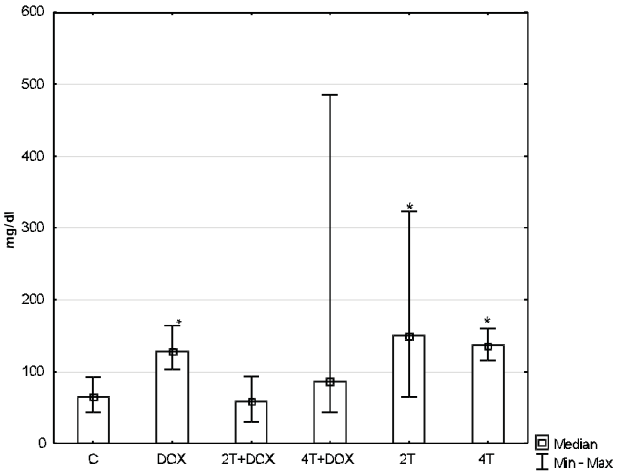
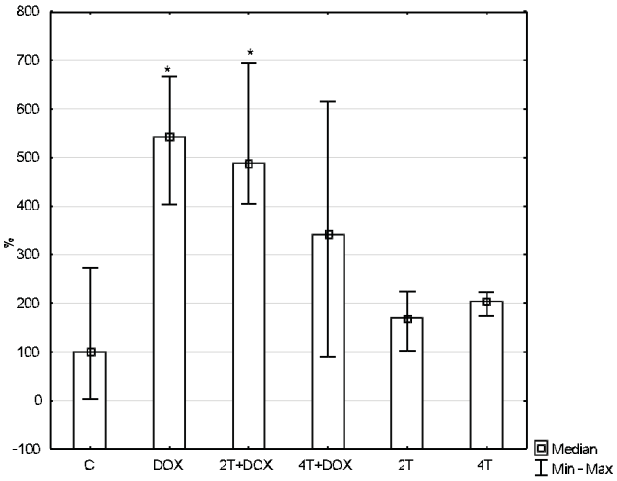


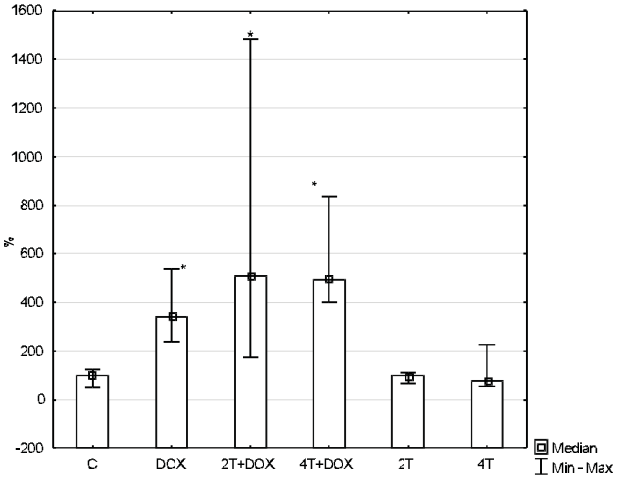
Fig. 1. Plasma glucose concentration [mg/dl]

GLUT4 mRNA concentration increased in the group receiving doxorubicin but thyroxine had no effect comparing to the control (Fig. 2). There were no significant statistical differences between groups administered with doxorubicin and doxorubicin with thyroxine.



* $p \leq 0.5$ vs. control

Fig. 2. GLUT4 mRNA concentration



* $p \leq 0.5$ vs. control

Fig. 3. GLUT4 protein concentration;

Similar results were found for GLUT4 protein (Fig. 3). There were no statistically important differences between doxorubicin and doxorubicin with thyroxine.

DISCUSSION

According to the current theory, the delayed cardiotoxicity observed in patients after completing doxorubicin therapy is related to oxidative stress in mitochondria [19]. Oxidative mtDNA damages result in synthesis of defective protein that augments oxidative damages in mtDNA. The repeating cycle of these events eventually manifests as insufficiency of mitochondrial respiration. As a result, the decreasing ATP synthesis caused by doxorubicin [20] may trigger adaptive processes in cardiomyocytes [19]. Glycolysis is an alternative but not as efficient as the mitochondrial pathway to gain ATP. In that case, increased glucose transport to cell is expected.

Results of our examinations confirmed the above assumptions. Doxorubicin in the cardiac muscle caused an increase in the amount of the transporter of the GLUT4 mRNA and protein. Moreover, blood glucose concentration was also increased in DOX group of rats. It is possible that because of mitochondria damages in cardiomycytes, β-oxydation is inhibited and secondary increase in activity of glycolysis increases cell request for glucose.

In a study by Hrelia *et al.* [8] there were no changes in cardiac GLUT4 level in rats receiving doxorubicin. In the case of isoform GLUT1 researchers demonstrated different results. The cause of this discrepancy probably results from different doses, number of application and the period of time from last doxorubicin administration to tissue examination. In the cited study, glucose capture by cells increased twice at 1h incubation with doxorubicin and with the passing of time (2-6 h), it was progressively inhibited to the total stop of glucose uptake. This effect may be connected with oxidative stress because antioxidants protect from this phenomena [8].

The assumption stated above dealing with inhibition of β-oxydation and increase of glucose utilisation was consistent with other studies [6, 19]. Interestingly, in a study by Abdel-Aleem *et al.* [1] it was found that the concentration of doxorubicin to inhibit β-oxydation is smaller than the concentration needed for glucose oxidation. Referring to blood glucose concentration Geetha *et al.* [6] observed an increase similar to our results after doxorubicin treatment to the rats. However, Delcers and Goormaghtigh [4] suggested that the rise in glucose level might result from spleen-toxicity effect, which is consistent with a study by Geetha *et al.* [6].

In the current study, it was shown that thyroxine had no significant effect on cardiac levels of GLUT4 mRNA and protein and blood glucose in rats receiving doxorubicin comparing to the group, which was administered only doxorubicin. The obtained results suggest that the demand for glucose was not changed between groups DOX and DOX+T4. Iodothyronin hormones affect the heart muscle cells metabolism for regulation genes responsible for the synthesis of the mitochondrial respiratory chain and oxidative phosphorylation enzymes [9]. On this basis, we expected activation of cell oxidation and a secondary increase of the demand for glucose. That thesis is consistent with a study by Braunwald *et al.* [2], who concluded that the higher metabolism the higher the demand for glucose and the more increased level of GLUT4. Loffer *et al.* [11] postulated that thyroxine-dependent increase might be attributed to gluconeogenesis glicogennolysis activation in liver. Simultaneously, iodothyronine hormones inhibit glycogen synthase [7] and increase glucose absorption from the digestive tract [21]. Interesting results were observed in DOX+4T4 group, where standard deviation was about 10-fold higher comparing to the control. It means that an individual reaction is highly divergent.

In conclusion, the present study confirmed that doxorubicin causes an increased demand for glucose as suggested by a higher cardiac level mRNA and protein for GLUT4. Thyroxine has no effect on these changes induced by doxorubicin.

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