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Short Communication

Differential response of oxygen radical metabolism in rat heart, liver and kidney to cyclosporine A treatment

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Abstract. *Objective and Design*: The study was designed to elucidate whether cyclosporine A (Cy A) induces oxidative stress in heart, liver and kidney.
Material and Treatment: Male Wistar rats were treated with NaCl (n = 7), cremophor (vehicle for Cy A; n = 7) and 30 mg/kg b.w. Cy A in cremophor (n = 7) daily for 4 weeks.
Methods: Oxidized (GSSG) and reduced (GSH) glutathione, lipid peroxides and superoxide dismutase were measured in the organs.
Results: Increases in GSSG [nmol/mg prot.] and a compensatory rise in total GSH [nmol/mg prot.] indicating Cy A-induced oxidative stress were found in kidney (0.39 ± 0.09 vs. 0.47 ± 0.14 vs. 0.64 ± 0.18; 20.71 ± 3.86 vs. 21.07 ± 3.86 vs. 28.14 ± 3.37) and liver (0.51 ± 0.11 vs. 0.51 ± 0.09 vs. 0.65 ± 0.25; 33.35 ± 5.06 vs. 32.88 ± 5.12 vs. 44.12 ± 6.06) but not in heart.
Conclusion: Cy A-induced oxidative stress may contribute to the hepatotoxicity and nephrotoxicity of this drug. After heart transplantation, accelerated allograft atherosclerosis limits transplantation success. We did not find any evidence that Cy A induces oxidative stress in the heart which might favour atherogenesis.

Key words: Cyclosporine A – Oxidative stress – Heart – Liver – Kidney

Introduction

Side-effects of cyclosporine A (Cy A), mainly affecting the kidney but also observed in liver, limit the therapeutic use of this drug after transplantation. It has been suggested that Cy A-induced oxidative stress could be responsible for the nephrotoxicity and hepatotoxicity of this drug [1–3]. Recently, Tatou et al. demonstrated an increased formation of lipid peroxides in isolated rat heart during ischemia/reperfusion experiments in the presence of Cy A [4]. On the other hand, oxygen radical-dependent reactions, such as lipid peroxidation are implicated in the initiation and progression of atherosclerotic processes [5]. Based on this, we hypothesized that long-term Cy A treatment might induce oxidative stress in the heart which could favour the development of accelerated atherosclerosis in the allograft heart, the major limiting factor for long-term survival of heart transplant recipients. This hypothesis was supported by the demonstration of increased levels of lipid peroxides in the plasma of patients with accelerated atherosclerosis [6].

To elucidate whether long-term, high-dose treatment with Cy A induces oxidative stress in heart, kidney and liver, we analyzed the tissue concentrations of oxidized (GSSG) and reduced glutathione (GSH), the tissue and plasma levels of lipid peroxide (LPO) and the tissue superoxide dismutase activities (SOD) of rats treated with Cy A.

Materials and methods

The experiments were conducted in accordance with the Guide for the care and use of laboratory animals (NIH publication 85-23). Male Wistar rats (230 ± 20 g body weight) were divided into three groups. Group 1 (control group, n = 7) was treated intraperitoneally with sodium chloride, group 2 (n = 7) with cremophor (vehicle for Cy A) and group 3 with 30 mg/kg body weight Cy A suspended in cremophor daily for 4 weeks. The rats were allowed free access to food and water. After 4 weeks, the animals were anaesthetized by intraperitoneal treatment

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with pentobarbital (25 mg/kg), heparinized and sacrificed by cervical dislocation. The organs were rapidly removed, rinsed in ice-cold physiological saline solution, blotted and immediately frozen in dry ice. Blood plasma was prepared from the heparinized blood and frozen. Organs and plasma were stored at −80 °C until analysis.

The tissues (50 mg/ml) were homogenized in isotonic NaCl at 4 °C. Aliquots of the homogenates were taken for the analytical procedures.

The GSH and GSSG concentrations were measured according to Beutler et al. [7] and Hissin and Hilf [8], respectively. To prevent GSH autoxidation 50 mM NEM was added. The plasma and tissue LPO concentrations were measured according to Ohkawa et al. [9] and Yagi [10], respectively.

Measurement of SOD activity was based on the method of Beauchamp and Fridovich [11] in terms of its ability to prevent the reduction of nitroblue tetrazolium (NBT) by superoxide radicals.

Protein concentration was measured according to Lowry et al. [12].

All results are expressed as means ± SD. Statistical analysis was done using ANOVA with Tukey test for intergroup comparison. Differences were considered to be significant at the level $p \leq 0.05$.

Results

All results are given in table 1 which shows that the Cy A treatment increased the GSH-levels in kidney and liver as well as the GSSG concentration, especially in kidney. The GSSG level tended to be higher in liver. In contrast, the Cy A treatment did not increase the GSH and GSSG levels in the heart. A lowering of GSSG concentration was even observed in the heart of the Cy A treated animals. The Cy A treatment had no effect on the LPO and SOD levels in the investigated organs.

Discussion

The GSSG increase found in kidney and liver of the Cy A treated animals indicates an elevated formation of oxygen radicals induced by Cy A in these organs. This GSSG increase could result from an accelerated activity of glutathione peroxidase necessary for the removal of lipid peroxides formed in these organs during the Cy A treatment.

On the other hand, it is known that the GSH system is able to adapt to chronic oxidative stress as shown by an increase in the total GSH concentration in the organs [13]. Because of this, the increased GSH levels found in liver and kidney after Cy A treatment may also be regarded as a sign of Cy A stimulated oxygen radical formation in these organs.

In spite of the increased GSH turnover in liver and kidney of Cy A treated rats, we did not find increased concentrations of LPO (typical marker of oxygen radical induced damage) which has been observed by others [3]. In such studies (in general using short-term Cy A treatment), the GSSG increase was accompanied by unchanged or decreased GSH levels. The resulting increased ratio of oxidized GSH to total GSH [1, 2] points towards an imbalance in oxygen radical metabolism after short-time Cy A treatment that could have facilitated the lipid peroxidation demonstrated in these studies. According to our study design, a similar but temporary effect of Cy A may have arisen immediately after onset of the treatment. However, the long-term Cy A treatment investigated in our study led to a compensatory GSH increase in liver and kidney. Therefore, the ratio of oxidized GSH to total GSH remained constant. This adaptation of liver and kidney to Cy A-induced oxygen radical formation, combined with the unchanged activity of enzymatic antioxidants (SOD) which prevented a disturbance of oxygen radical metabolism, could have been responsible for the protection of liver and kidney against lipid peroxidation. We assume that an excessive prolongation of the Cy A treatment could exhaust the endogenous mechanisms of adaptation against oxidative stress in liver and kidney. Therefore, additional antioxidant supplementation as suggested by Wang et al. [3] should be helpful.

Table 1. Plasma levels of lipid peroxides [nmol/ml] and concentrations of lipid peroxides [nmol/mg prot.], GSH [nmol/mg prot.], GSSG [nmol/mg prot.], total glutathione (2GSSG + GSH) [nmol/mg prot.] and ratios of ox. GSH/total glutathione (2GSSG/2GSSG + GSH) [%] and SOD activities [U/mg prot.] in heart, liver and kidney of rats following daily i.p. treatment of Cy A (30 mg/kg b.w. suspended in cremophor), cremophor and NaCl for four weeks. $^{\times}p \leq 0.05$ (NaCl vs. Cy A); $^{+}p \leq 0.05$ (Cremophor vs. Cy A).

	NaCl n = 7	Cremophor n = 7	Cyclosporine A n = 7
Plasma			
Lipid peroxide	13.7 ± 1.2	14.2 ± 1.4	14.1 ± 1.3
Heart			
Lipid peroxide	0.99 ± 0.12	0.98 ± 0.20	1.12 ± 0.11
GSH	12.92 ± 4.67	11.25 ± 3.33	11.58 ± 2.33
GSSG	0.56 ± 0.14	0.62 ± 0.18	0.36 ± 0.09 [×;+]
2GSSG + GSH	14.00 ± 6.58	12.42 ± 3.42	12.33 ± 1.92
2GSSG/2GSSG + GSH	7.97 ± 2.02	9.93 ± 2.95	5.81 ± 1.47 [+]
SOD	11.70 ± 2.05	10.50 ± 2.50	12.05 ± 2.55
Liver			
Lipid peroxide	1.11 ± 0.18	1.19 ± 0.25	1.19 ± 0.15
GSH	32.35 ± 4.24	31.88 ± 4.71	42.82 ± 5.76 [×;+]
GSSG	0.51 ± 0.11	0.51 ± 0.09	0.65 ± 0.25
2GSSG + GSH	33.35 ± 5.06	32.88 ± 5.12	44.12 ± 6.06 [×;+]
2GSSG/2GSSG + GSH	3.03 ± 0.32	3.11 ± 0.54	2.93 ± 0.12
SOD	18.55 ± 2.85	17.50 ± 1.80	19.55 ± 1.50
Kidney			
Lipid peroxide	1.25 ± 0.42	1.17 ± 0.19	1.30 ± 0.13
GSH	19.93 ± 3.92	20.14 ± 3.43	26.86 ± 3.37 [×;+]
GSSG	0.39 ± 0.09	0.47 ± 0.14	0.64 ± 0.18 [×]
2GSSG + GSH	20.71 ± 3.86	21.07 ± 3.86	28.14 ± 3.37 [×;+]
2GSSG/2GSSG + GSH	3.79 ± 0.83	4.33 ± 1.35	4.56 ± 1.27
SOD	12.50 ± 1.85	12.00 ± 2.05	13.50 ± 1.75

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In contrast to the situation in liver and kidney, after the Cy A treatment we found no GSSG increase and no signs of adaptation of the GSH system to increased oxygen radical formation in the heart. These findings argue against Cy A induced stimulation of oxygen radical formation in the heart. This is in contrast to the study of Tatou et al. [4], who found increased lipid peroxidation in isolated heart if a cycle of acute ischemia/reperfusion was performed in the presence of Cy A. However, the model they used to analyze acute effects of Cy A is completely different to our model of long-term Cy A treatment. Tatou et al. [4] suggested also that cremophor intensifies the toxic effects of Cy A on the heart. But neither the results of the study of Tatou et al. [4] nor of our study indicate that the more pronounced toxic effects of the combination of cremophor and Cy A are caused by greater stimulation of oxygen radical formation.

With regard to the mechanisms by which Cy A might form oxygen radicals, cytochrome P 450 is regarded as the most important oxygen radical source during Cy A treatment [14, 15]. The activity of cytochrome P 450 is very different in liver, kidney and myocardium. Whereas cytochrome P 450 activity in liver and kidney of mammals, including human is high, the myocardium is characterized by a very low activity of this drug metabolizing system [16, 17]. The different Cy A effects in the heart compared with those we found in liver and kidney might be due to these organ-specific differences in cytochrome P 450 activity.

Based on our findings, we suggest that Cy A treatment is associated with stimulation of oxygen radical formation in liver and kidney. In this way long-term treatment with Cy A may contribute to the toxic side-effects of the drug in these organs. We found no evidence for specific Cy A-induced oxygen radical formation in the heart that could facilitate accelerated cardiac atherosclerosis as a limiting factor for long-term survival of heart transplant recipients.

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