Superoxid dismutase activity in portal vein endothelium after partial liver resection[1]

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ABSTRACT

PURPOSE: To investigate superoxide dismutase (SOD) activity in the portal vein endothelium and malondialdehyde acid (MDA) production in liver tissue of rats submitted to 70% hepatectomy.

METHODS: Twelve rats were distributed in two groups (hepatectomy and sham). Animals were sacrificed on post operative day 1 and portal vein, liver tissue and blood samples were collected. Portal vein SOD production was measured using lucigenin-amplified chemiluminescence assays**.** MDA measurement was used as an index of oxidative stress through the formation of TBARS (Thiobarbituric Acid Reactive Species).

RESULTS: There was no difference in post operative bilirrubin, AST, ALT levels between groups. DHL level was higher in the hepatectomy group ($p=0.01$). MDA production in the remnant liver tissue and endothelial portal vein SOD activity were also significantly ($p<0.05$) elevated in the hepatectomy group when compared to control group. There was no correlation between MDA and SOD activity. SOD activity, on the other hand, showed a positive correlation with LDH level ($p=0.038$) and MDA levels showed a positive correlation with AST and ALT levels ($p<0.001$).

CONCLUSION: There is an increased production of malondialdehyde acid in liver tissue after partial hepatectomy and increased activity of superoxide dismutase in portal vein endothelium as well.

Key words: Hepatectomy. Oxidative Stress. Malondialdehyde. Superoxide Dismutase. Rats.

Introduction

Liver regeneration is a multi step process that activates quiescent hepatocytes through a complex biochemical pathway, beginning with an inflammatory process followed by cellular replication[1-2]. When the initial inflammatory response is insufficient (as when less than 10% of liver parenchyma is removed) the starter mechanism is not triggered and regeneration is not initiated; on the other hand, when too much liver is resected, an over inflammatory response is activated and the initial essential inflammatory process turns into deleterious. This situation is well known as small for size graft in liver transplantation and measurements to control the inflammatory cascade can be applied, as porto-cava shunt, splenectomy or splenic artery embolization/ligation[3-4].

In this circumstance, portal hemodynamic and venous distension play an essential role, once acute portal hypertension can cause endothelial damage, condition known as shear stress[5-7]. There are few studies measuring the splancnic endothelial damage (directly or indirectly, measuring – ROS - production) and some of them report that endothelial cells play an important role in modulating this complex mechanism, balancing the portal pressure and the local inflammatory response[7-10]. Super oxid dismutase is an enzyme that catalises transformation of superoxide into H2O2 and O2 and is over produced in stress situations, like portal hypertension and ischemic/reperfusion models[7,11]. As liver tissue is immersed in a complex capillary system, measurement of malondialdehyde acid (MDA) in liver tissue can also reflect the inflammatory response that takes place after partial liver resection[12-14].

In the English medical literature we could not find any report of endothelial superoxide dismutase (SOD) portal vein measurement after partial hepatectomy and its correlation with ROS production (MDA) in liver tissue. This motivated us to conduct this study.

The aim of the article is to evaluate SOD activity in the portal vein endothelium and MDA production in liver tissue of rats submitted to 70% hepatectomy.

Methods

The experiments were approved and registered by Research Ethical Committee, Medical School,USP, (process number 0991/07)

Male Wistar rats weighting from 250 to 300g were operated after intraperitoneal cetamine anesthesia (cetamine chloridrate, 10%, 0.1ml/100g of weight). Standard liver resection (70%) as proposed by Higgins and Anderson was applied. Animals were divided in two groups: 1-control, in which laparotomy was performed followed by closure (post operative day POD-0) and portal vein resection on POD1 and 2- hepatectomy, in which liver resections was performed (POD0) and portal vein resection was done on POD1. Resected liver tissue and rat's weight were measured.

To assess damage to the hepatic parenchyma, levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and bilirubin were measured using a serum analyzer (spectophotometric determination of plasma levels). Portal vein was resected carefully below its bifurcation until the upper border of duodenum. ROS production was evaluated measuring portal vein endothelium superoxide production.

Portal vein superoxide dismutase production was measure using lucigenin-amplified chemiluminescence assays. Each rat portal vein segment was immersed in Krebs buffer at pH 7.40, for 20 min. The vein segments were then rapidly transferred to a counting vial under light protection and immersed in a 5-μM lucigenin (Sigma, USA) solution in Krebs buffer (total volume = 1.0 mL). This lucigenin concentration is within the range proposed to be less likely to undergo redox cycling, reflecting, therefore, superoxide generation by tissues. The luminescence emitted by each fragment was measured for 5 min in a Berthold Multi Biolumatluminometer (Berthold, Germany). Background signals from buffer and lucigenin were subtracted from vein signals and the resulting value was normalized for dry weight. Data are reported as counts per min (cpm) per mg of dry tissue. Chemiluminescence was measured directly and continuously for 5 min to obtain basal values. Superoxide generation from portal vein segments was obtained under several experimental conditions. To analyze the role of specific pathways, some compounds were added at the following final concentrations: 1) 1 mM L-NAME, 2) 10 μM reactive blue, 3) 1 μM pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS), 4) 0.1 mM 2-methylthioadenosine 5'-triphosphate (2MeSATP; a non-selective P2Y agonist), and 5) 30 μM β-γ-methylene-ATP (a partially selective P2X antagonist). After 5 min a new record of superoxide production was obtained. ATP was added at a final concentration of 0.1 mM, 10 min after addition of the compounds, and after 5 min a new record of superoxide production was obtained.

Malondialdehyde acid measurement: liver tissues were immediately kept at -70°C until assayed for thiobarbituric acid reactive species (TBARS) formation. As an index of oxidative stress we used the formation of TBARS (Thiobarbituric Acid

Reactive Species) during an acid-heating reaction. Briefly, the samples were mixed with 1 mL of trichloroacetic acid 10% and 1 mL of thiobarbituric acid 0.67% and then heated in a boiling water bath for 15 min. TBARS was determined by the absorbance at 535 nm using 1,1,3,3-tetramethoxypropane as an external standard. Results are expressed as malondialdehyde equivalents per milligram of protein (Lowry assay).

Results

There was no difference in rat's weight between groups. Mean of 7.67g of liver was resected. Bilirrubin, AST, ALT and LDH mean, standard deviation and range are depicted in Table 1.

TABLE 1 - Mean, standard deviation, range of the variable analyzed and comparison between groups.

Variable	Group	N	Mean	Std. Deviation	Minimum	Maximum	p
Weight	Hepatectomy	6	251.02	18.4	230.4	269.1	0.09
	Control	6	272.93	21.9	243.6	301.3	
	Total	12	260.34	25.7	249.6	282.6	
AST	Hepatectomy	6	367.17	681.5	44	1757	0.43
	Control	6	144.00	60.3	81	245	
	Total	12	255.58	475.7	44	1757	
ALT	Hepatectomy	6	201.33	207.2	73	621	0.09
	Control	6	46.67	9.0	37	59	
	Total	12	124.00	161.5	37	621	
BD	Hepatectomy	6	1.23	1.29	.2	3.3	0.18
	Control	6	.45	.37	.2	1.2	
	Total	12	.84	.99	.2	3.3	
BT	Hepatectomy	6	1.81	1.75	.4	4.7	0.19
	Control	6	.76	.51	.4	1.8	
	Total	12	1.29	1.35	.4	4.7	
LDH	Hepatectomy	6	2962.67	1971.21	821	6660	0.01
	Control	6	492.83	297.05	239	1002	
	Total	12	1727.75	1862.79	239	6660	
MDA	Hepatectomy	6	4.78	3.03	2.8	10.8	0.04
	Control	6	2.07	.64	1.5	3.2	
	Total	12	3.42	2.52	1.5	10.8	
SOD	Hepatectomy	6	1390.2	851.8	693	3026	0.03
	Control	6	468.3	273.2	263	995	
	Total	12					

LDH levels were significantly (p=0.01) higher in the hepatectomy group when compared to control group, as seen in Figure 1.

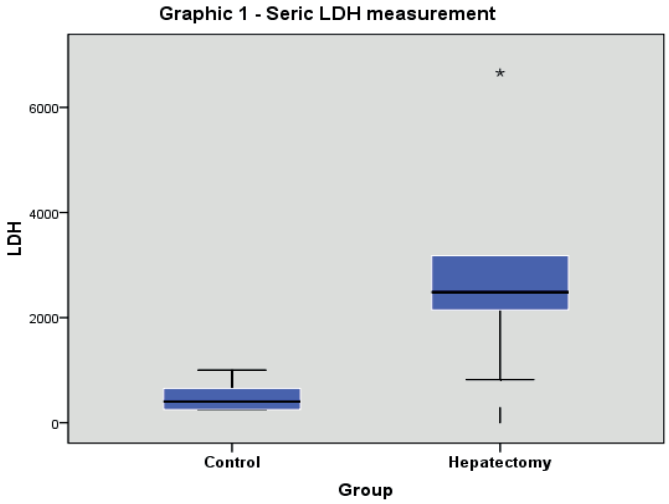

FIGURE 1 - Lactate dehidrogenase (LDH) seric measurement in the control and hepatectomy groups (p<0.05).

MDA production was also increased in the hepatectomy group (p=0.04) as seen in Figure 2.

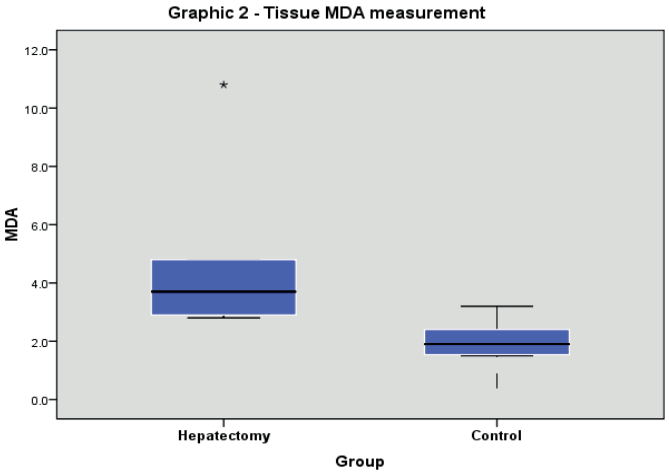

FIGURE 2 – Malondialdehyde acid (MDA) liver tissue measurement in the control and hepatectomy groups (p<0.05)

Superoxide activity was significantly (p<0.05) elevated in the hepatectomy (1536±310) group when compared to control group (629±110) as shown in Figure 3. SOD activity showed a positive correlation with LDH level (p=0.038 Pearson correlation of 0.65).

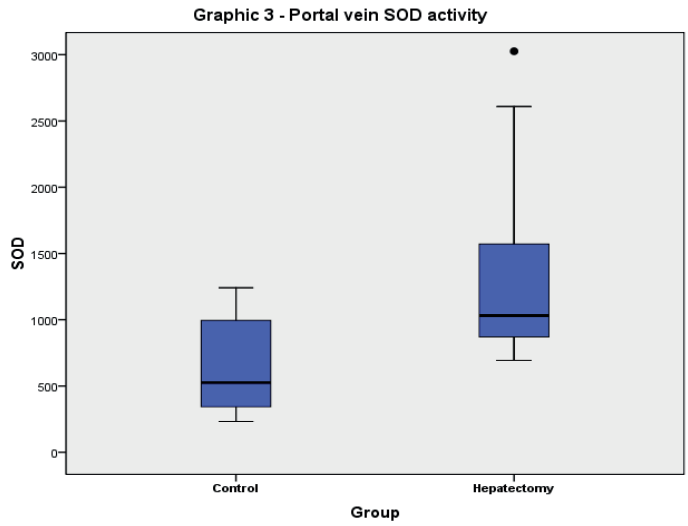

FIGURE 3 - Superoxid dismutase (SOD) activity in portal vein endothelium in the control and hepatectomy groups (p<0.05).

There was no correlation between SOD activity and MDA production (p=0,052). On the other hand, MDA levels showed a positive correlation with AST and ALT levels (p<0.001 in both cases, Pearson correlation of 0.89 and 0.94, respectively).

Discussion

Liver resection is safe with low morbidity and mortality rates in specialized center[15-16]. With peri operative improved management, such as portal vein embolization and tumor downstaging, extended resections can be performed[17]. But in certain circumstances, even after adequate work up, surgeons can face situation with small liver remnant. In this dangerous scenario, portal hemodynamics tries to overcome the new acute portal hypertension state by modulating the splancnic inflow, mainly by arterial vasoconstriction. But as portal circulation does not have constriction capacity, the same blood flow that was supposed to flow through normal size liver parenchyma gets into a small amount of hepatic tissue (liver remnant), tearing sinusoids, damaging endothelial cells and creating a super inflammatory response[4]. This response is deleterious to the liver regeneration process and the main mechanisms involved in its physiopathology are shear stress response, neutrophilic migration and endothelial damage[7-8,18-19].

In this setting, endothelial damage can occur by different mechanisms (neutrophilic migration, NADPH oxidation, activation of mieloperoxidase)[20-21], leading to super production of free radicals and pro inflammatory citoquines (IL-6/INF alfa) and finally cell death[22-24].

Endothelial over production of ROS can lead to activation of enzymes in order to modulate and buffer the deleterious effects of oxidation[25-27].

MDA, one of the products of lipid peroxidation, was used as a measurement of liver tissue damage and indirect reflects the intensity of cellular injury and oxygen derived free radicals production. MDA levels constitute a sensitive marker, reliably assessing the injurious effect of oxidative stress on various cellular membranes. MDA not only reflects the level of oxygen free radicals, but also can reflect the degree of liver cell damage. As demonstrated, partial liver resection increase the lipid peroxidation in liver tissue, also confirmed by others[28]. Positive correlation of MDA with AST and ALT reflects probably hepatocyte injury (higher levels of AST/ALT reflects on higher levels of MDA production).

Some studies have shown shear stress mediated up regulation of Cu/Zn SOD as an important endothelial cellular defense mechanism contributing to the inhibition of activation of the caspase cascade[13]. Thus, scavenging of O_2^- by the upregulation of Cu/Zn SOD by shear stress completely prevented TNF-α-induced apoptosis and increased the resistance to pro-apoptotic stimuli involving oxidative damage in endothelial cells[26,29]. As liver tissue is composed of sinusoidal cells surrounded by hepatocytes, excessive endothelial damage directly affects liver cells and could impair liver regeneration.

As demonstrated in our study, after partial hepatectomy portal vein endothelium produces more free radical than the control group, leading to a pro inflammatory status. This situation is well balanced and when over expression of pro inflammatory mediators occurs, measurements to overcome can be surgically (splenic artery ligation, portacaval shunt) or biochemically attempted. This physiopathology open opportunities to use drugs that could modulate the inflammatory response or could diminished the toxicity of cell products, as free radical scavengers.

Conclusions

Partial liver resection leads to an increase in superoxide dismutase (SOD) production by endothelial cells in the portal vein and in malondialdehyde acid (MDA) production by liver tissue. There is no correlation between MDA production and SOD activity.

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