Riboflavin (vitamin B-2) reduces hepatocellular injury following liver ischaemia and reperfusion in mice

Sheila Cristina Sanches a, Leandra Naira Z. Ramalho a, Mariana Mendes-Braz a, Vânia Aparecida Terra b, Rubens Cecchini b, Marlei Josiele Augusto a, Fernando Silva Ramalho a,⇑

a Department of Pathology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
b Department of Pathological Sciences, State University of Londrina, Londrina, PR, Brazil

ABSTRACT

Riboflavin has been shown to exhibit anti-inflammatory and antioxidant properties in the settings of experimental sepsis and ischaemia/reperfusion (I/R) injury. We investigated the effect of riboflavin on normothermic liver I/R injury. Mice were submitted to 60 min of ischaemia plus saline or riboflavin treatment (30 μmoles/kg BW) followed by 6 h of reperfusion. Hepatocellular injury was evaluated by amino-transferase levels, reduced glutathione (GSH) content and the histological damage score. Hepatic neutrophil accumulation was assessed using the naphthol method and by measuring myeloperoxidase activity. Hepatic oxidative/nitrosative stress was estimated by immunohistochemistry. Liver endothelial and inducible nitric oxide synthase (eNOS/iNOS) and nitric oxide (NO) amounts were assessed by immunoblotting and a chemiluminescence assay. Riboflavin significantly reduced serum and histological parameters of hepatocellular damage, neutrophil infiltration and oxidative/nitrosative stress. Furthermore, riboflavin infusion partially recovered hepatic GSH reserves and decreased the liver contents of eNOS/iNOS and NO. These data indicate that riboflavin exerts antioxidant and anti-inflammatory effects in the ischaemic liver, protecting hepatocytes against I/R injury. The mechanism of these effects appears to be related to the intrinsic antioxidant potential of riboflavin/dihydroriboflavin and to reduced hepatic expression of eNOS/iNOS and reduced NO levels, culminating in attenuation of oxidative/nitrosative stress and the acute inflammatory response.

1. Introduction

Liver ischaemia is an inherent condition to hepatic transplantation as well as a surgical strategy often used during major hepatic resection surgeries. On restoring the blood supply, the liver is subjected to additional damage, exacerbating the injury previously induced by ischaemia. Liver injury secondary to ischaemia and reperfusion (I/R) is a serious problem affecting hepatic transplantation outcomes and represents the main cause of both poor function and primary non-function of liver allografts. Therefore, I/R injury has resulted in the search for methods that are able to preserve the functional and morphological integrity of transplanted livers (Casillas-Ramírez et al., 2006).

The excessive hepatic inflammatory response and tissue oxidant stress that result from the massive production and release of reactive oxygen species (ROS) by resident and infiltrating phagocytes are recognised as fundamental events in the pathophysiology of hepatic I/R injury. After endothelial transmigration, activated neutrophils induce cell death through the release of ROS and proteases into the liver interstitium. In addition to causing direct cellular damage through protein degradation, lipid peroxidation, and DNA damage, ROS can modulate the production of inflammatory mediators and adhesion molecules and support protease activity through the inactivation of anti-proteases (Jaeschke, 2003, 2006).

Riboflavin (vitamin B-2) is an essential water-soluble vitamin present in a wide variety of foods. Its most important biologically active forms, flavin adenine dinucleotide (FAD) and flavin mononucleotide, participate in a range of redox reactions, some of which are essential to the function of aerobic cells (Powers, 2003). Previous studies have shown that riboflavin can protect tissues from oxidative injury. In a murine heart transplantation model, riboflavin reduced myocardial lipid peroxidation, leukocyte infiltration, cytokine production, and cardiac allograft vasculopathy (Iwanaga et al., 2007). Riboflavin has also been reported to have a protective effect on the rat brain after ischaemia (Betz et al., 1994), on rabbit...
myocardium during re-oxygenation (Mack et al., 1995), and on the rat lung after I/R of the hind limbs (Seekamp et al., 1999). Moreover, riboflavin has been found to increase the survival rate of mice suffering endotoxin-induced sepsis and Gram-negative and Gram-positive bacterial infection. This beneficial effect on murine sepsis appears to be associated with the riboflavin-induced increased expression of heat shock protein 25 in the heart and lung (Toyosawa et al., 2004; Shih et al., 2010). Thus, we hypothesise that riboflavin can protect the liver from I/R damage. To prove this hypothesis, we investigated the effect of riboflavin in a normothermic I/R model in the mouse liver.

2. Materials and methods

2.1. Experimental animals

Male Swiss mice weighing 25–30 g were obtained from the Central Animal House of the University of São Paulo (Ribeirão Preto, Brazil). The mice were housed at 23 ± 2°C with a 12 h light/dark cycle, and were fed ad libitum with standard chow. This experimental protocol was approved by the Animal Research Ethical Committee of the School of Medicine of Ribeirão Preto-USP (process no. 44/2011).

2.2. Surgical procedure

After anaesthesia was induced with ketamine (120 mg/kg BW) and xylazine (5 mg/kg BW), median laparotomy was performed and the blood supply to the left lateral and median lobes of the liver was interrupted for 60 min by placing a microvascular clamp across the portal triad to these lobes. Contraction of the bowel was avoided during the clamping period by preserving the portal flow through the right lateral and caudate lobes. At the end of the ischaemia period, reperfusion was initiated by releasing the clamp. (Mendes-Braz et al., 2012) Mice were re-anesthetised with ketamine/xylazine 6 h after reperfusion for sample harvesting.

2.3. Experimental design

The animals were divided at random into three experimental groups (n = 6–8 per group) as follows: mice subjected to the surgical procedure described above and treated with riboflavin (10 μmoles/kg BW – I/R RIBOFLAVIN group) or saline solution (I/R group) administered intraperitoneally 30 min before ischaemia, 30 min after ischaemia and 1 h after reperfusion and sham-operated mice (SHAM group). A preliminary pilot study was performed in mice with different doses of riboflavin to determine the riboflavin dose with the maximum tolerability and efficacy. At the time of sacrifice, venous blood samples were collected under anaesthesia, and the serum was stored at −80°C. Liver tissue specimens from the median and left lateral lobes were harvested and frozen at −80°C or fixed in 4% neutral formalin and embedded in paraffin blocks.

2.4. Measurement of serum aminotransferases

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured spectrophotometrically in serum using a commercial kit according to manufacturer's instructions (LabTest, Lagoa Santa, Brazil) at an absorbance of 340 nm.

2.5. Histological assessment of liver injury

Formalin-fixed and paraffin-embedded tissue sections were cut to a thickness of 5 μm and stained with haematoxylin and eosin for histological examination. A pathologist who was blinded to the treatment used light microscopy to assess the degree of liver damage. Analysis was performed on 30 randomly chosen high-power fields (HPFs; 400× magnification) in each slide. Grading of the severity of hepatic injury was as follows: grade 0, minimal or no evidence of injury; grade 1, mild injury consisting of cytoplasm vacuolization and focal nuclear pyknosis; grade 2, moderate to severe injury with extensive nuclear pyknosis, cytoplasmic hemozoin formation and loss of intercellular borders; and grade 3, severe necrosis with distortion of hepatocellular cords, haemorrhage, and neutrophil infiltration (Serafin et al., 2002).

2.6. Histological evaluation of hepatic neutrophil infiltration

Infiltration of neutrophils into the liver was estimated using the naphthol AS-D chloroacetate esterase staining method, which identifies specific leucocyte esterases (Moloney et al., 1960). Briefly, the 5 μm paraffin sections were deparaffinised with xylene, rehydrated through an alcohol series, and then immersed in distilled water before being incubated in naphthol esterase solution at room temperature for 15 min. Naphthol esterase solution contains Naphthol AS-D chloroacetate (Sigma Chemical Co., St. Louis, MO. U.S.A.) in N,N-dimethyl formamide (2 mg/mL), 4% sodium nitrite, and 4% New Fuchsin in 2 N HCl combined in 0.1 M phosphate buffer (pH 7.6). The sections were rinsed with tap water and counterstained with Gill's hematoxylin for 15 s. Red color was deposited only in neutrophils and mast cells. The identification of the stained neutrophils was made based on nuclear morphology and red small granular deposits scattered within the cytoplasm. Only polymorphonuclear cells (PMN) located within sinusoids or extravasated into the surrounding parenchyma and characterized with a multilobed nucleus and red granular deposits within the cytoplasm were counted. The number of esterase-positive PMNs was counted in 30 HPF (400× magnification) in each sample, and mean values were calculated.

2.7. Assessment of neutrophil infiltration by hepatic myeloperoxidase activity

The extent of neutrophil accumulation in liver tissue was also measured using a myeloperoxidase (MPO) activity assay as previously described (Ivey et al., 1995). Briefly, liver samples (100 mg tissue per 2 mL buffer) were homogenised in buffer (100 mM NaCl, 20 mM NaH2PO4, 15 mM NaEDTA, pH 4.7) and centrifuged at 3000 rpm for 10 min. The pellet then underwent hypotonic lysis (5 mL 0.2% NaCl) by 30 s followed by the addition of an equal volume of solution containing 1.6% NaCl and 5% glucose. After further centrifugation, the pellet was resuspended in 0.05 M NaH2PO4 buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide (HTAB, Sigma Chemical Co.). One-millilitre aliquots of the supernatant were transferred into 1.5 mL tubes followed by three freeze–thaw cycles using liquid nitrogen. The samples were centrifuged for 12 min at 4000 rpm, and the supernatants were used for MPO measurements. MPO activity was assayed by measuring the change in optical density at 450 nm using tetramethylbenzidine (1.6 mM) and H2O2.

2.8. Measurement of hepatic reduced glutathione and superoxide dismutase activity

To measure the levels of reduced glutathione (GSH) liver samples were homogenised in 1:11 KCl, and proteins were precipitated with 1% perchloric acid. After centrifugation, samples were neutralised with 10% K2CO3. GSH was measured using glutathione transferase and 1-chloro-2,4-dinitrobenzene. A 50 mL aliquot of the sample was mixed with 225 mL of 0.1 M potassium phosphate buffer, pH 7.0 and 10 mL of 10 mM 1-chloro-2,4-dinitrobenzene in ethanol. The reaction was started with 5 mL of glutathione transferase solution (12 U/L) and monitored at 340–400 nm, reaching the endpoint 5 min after enzyme addition (Brigelius et al., 1983). Superoxide dismutase (SOD) activity was measured in the liver according to Marklund and Marklund (1974), based on the inhibition of pyrogallol autoxidation in an aqueous solution of SOD. This oxidation is accompanied by a yellow color formation in the reaction medium, monitored at 420 nm. Aliquots of supernatant liver homogenate (20 mg tissue/mL) diluted in Tris buffer with 1 N HCl and 5 mM EDTA, pH 8.0, was added to pyrogallol. The reaction was monitored continuously for 5 min. The autoxidation of pyrogallol alone was used as control. One SOD activity unit (U) was defined as the enzyme amount causing 50% inhibition of pyrogallol autoxidation. Final results of hepatic GSH levels and SOD activity were expressed in nmol/mg protein and U/mg protein, respectively.

2.9. Nitric oxide quantification by luminol-H2O2-induced chemiluminescence assay

NO was measured as described by Kikuchi et al. (1991) with the following modifications. Rat liver samples (2.5 mg/mL) were prepared under N2 bubbling to ensure O2-free medium in 2 mM Na2EDTA buffer, at pH 8.5, which was previously degassed by N2, for 45-s periods of homogenisation. Equal volumes of 360 μM luminol/3 mM desferrioxamine (DFO) and 200 mM H2O2 were mixed and incubated at room temperature under moderate agitation for 5 min. To initiate the chemiluminescence reaction, 50 μL of this mixture was added automatically in the luminometer chamber containing a final volume of 850 μL. The chemiluminescence spectrum was recorded for 5 min using a GLOMAX TD/20 20 luminometer (Turner Designs, USA). Origin v.7.5 software was used to plot chemiluminescence curves that were analysed using the peak height (PMH) and AUC to determine the NO present in the sample. The results were expressed in relative light units/g tissue (NO RLU/g tissue). To prove the assay sensitivity to NO quantification, 100 μM 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (cPTIO), a specific scavenger of NO, was added immediately before the quantification of light emission in chemical (standard NO solution) and biological (liver sample) analyses (Terra et al., 2012).

2.10. Immunohistochemical detection of nitrotyrosine and 4-hydroxynonenal adducts

Four-μm-thick paraffin-embedded liver sections mounted on poly-1-lysine-coated slides were deparaffinised, rehydrated, immersed in 10 mM/L citrate buffer, pH 6.0, and submitted to heat-induced epitope retrieval using a vapour lock for 45 min. After heating, the slides were allowed to cool to room temperature and were briefly washed with Tris-buffered saline solution. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 5 min. Normal serum (Novostain Super ABC kit; Novocastra Laboratories, Newcastle Upon Tyne, U.K.) was applied for 30 min to block non-specific binding. For nitrotyrosine immunostaining, sections were blocked with 5% normal goat serum in PBS. The sections were incubated for 1 h at room temperature with a rabbit polyclonal antibody to 3-nitrotyrosine (1:500, Santa Cruz Biotechnology, USA). Sections were then rinsed with PBS, incubated with biotinylated secondary antibodies (ABC kit; Vector Laboratories, Peterborough, UK) for 30 min at room temperature followed by the addition of the avidin-biotin-peroxidase complex (20 min, 37°C). Sections were washed and then incubated with a solution containing 20 μg/mL diaminobenzidine (DAB) and 0.02% hydrogen peroxide in 0.05 M Tris buffer (pH 7.6). Then, the sections were rinsed with tap water and counterstained with 0.05% (w/v) hematoxylin for 1 min, followed by dehydration and mounting with cover slips.
United Kingdom) was used for 30 min to block nonspecific immunooasaying. Immunohistochemical staining was performed using an avidin–biotin peroxidase system (Novocastra Laboratories). The sections were then incubated with a monoclonal anti-4-hydroxynonenal (4-HNE) adduct antibody (A.G. Scientific Inc., San Diego, CA, U.S.A.) or a monoclonal anti-nitrotyrosine antibody (HM11; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) as the primary antibody, diluted 1:100, for 1 h at room temperature (approximately 25 °C) in a humidified chamber. After washing in phosphate-buffered saline a biotinylated universal secondary antibody (Novocastra Laboratories) was applied for 30 min. The sections were incubated with the avidin–biotin complex reagent (Novocastra Laboratories) for 30 min and developed with 3,3-diaminobenzidine tetrahydrochloride in phosphate-buffered saline, pH 7.5, containing 0.036% hydrogen peroxide, for 5 min. Light Mayer’s hematoxylin was applied as a counterstain. The slides were then dehydrated in a series of ethanol dilutions and mounted with Permount (Fischer, Fairlawn, NJ, U.S.A.). An analysis was performed on 30 randomly chosen HPFs (400× magnification). Immunoreactivity to 4-HNE and nitrotyrosine was evaluated as the percentage of positive points/HPF.

2.1. Detection of endothelial and inducible nitric oxide synthases by Western blotting

Liver samples (100 mg) were homogenised in 1 mL of lysis buffer [20 mM EDTA, 1% Triton, 0.1% SDS, 10 mM NaF, 1 mM Na₂VO₄, 10 mM glycerophosphate and protease inhibitors (one tablet per 10 mL buffer, Complete, Roche)] for 30 s. The suspensions were incubated on ice for 15 min and centrifuged at 10,000 rpm at 4 °C for 20 min. Protein concentrations were measured using the Bradford colourimetric method. Then, 30 μg protein was resolved by electrophoresis on 10% SDS-polyacrylamide gels, and blotted onto PVDF membranes (Amersham Life Science, Arlington Heights, IL, U.S.A.). After blocking with TBS solution (pH 7.4) containing 0.05% Tween 20 and 5% fat free milk for 1 h at room temperature, the membranes were incubated “overnight” at 4 °C with primary monoclonal anti-endothelial nitric oxide synthase (eNOS), anti-inducible nitric oxide synthase (i-NOS), or anti-β-actin antibodies (Santa Cruz Biotechnology). Bands were detected using a chemiluminescence system with HRP-conjugated secondary antibodies and ECL-plus reagents (Amersham Life Science). Molecular weight markers were used to determine protein size (Sigma–Aldrich Co.), and β-actin was used as an internal control.

2.12. Statistical analysis

Data were analyzed using the GraphPad Prism software 4.0 (GraphPad Software, San Diego, CA, U.S.A.). All data are reported as mean ± S.E.M. Statistical comparisons of the groups were performed by nonparametric Kruskal–Wallis one-way analysis of variance followed by Dunn’s posttest or Mann–Whitney test. Probability values less than 0.05 were considered to be statistically significant.

3. Results

3.1. Riboflavin reduces hepatocellular injury following liver I/R

Fig. 1A–C shows the hepatic histological findings in the SHAM, I/R, and I/R Riboflavin groups. Both the serum aminotransferase levels (ALT and AST) and the parenchymal damage score were considerably augmented following hepatic normothermic I/R in saline-treated mice (2016.0 ± 484.0, 1840.0 ± 501.0, and 1.85 ± 0.23, respectively) in comparison to SHAM-operated mice (92.0 ± 11.0, 358.0 ± 74.0, and 0.0 ± 0.0, respectively) (Fig. 2G–I). Nitrotyrosine immunoreactivity was substantially enhanced in the livers subjected to I/R of saline-treated mice (35.37 ± 7.26) when compared with the livers of SHAM-operated mice (0.014 ± 0.001) (P < 0.01). In contrast, liver tissue samples from riboflavin-treated animals showed decreased nitrotyrosine staining (8.05 ± 1.75) (P < 0.05) (Fig. 2). To elucidate the mechanisms underlying this beneficial effect, we investigated whether riboflavin could modulate hepatic NO synthesis. Our results indicated that the livers of riboflavin-treated mice exhibited significantly decreased NO levels in relation to the I/R group (P < 0.01) (Fig. 2K). Then, we assessed the hepatic content of NOS by Western blotting, and it was found that riboflavin also reduced the levels of both eNOS and iNOS in the livers subjected to I/R and treated with riboflavin when compared with those treated with saline (Fig. 2L).

3.2. Riboflavin suppresses neutrophil accumulation in the liver

Photomicrographs of naphthol chloroacetate staining, a specific marker of tissue nitrosative stress mediated by peroxynitrite and NO, are shown in Fig. 2G–I. Nitrotyrosine immunoreactivity was substantially enhanced in the livers subjected to I/R of saline-treated mice (35.37 ± 7.26) when compared with the livers of SHAM-operated mice (0.014 ± 0.001) (P < 0.01). However, the hepatocellular reserves of GSH were partially recovered by riboflavin administration (0.136 ± 0.011) (P < 0.05) (Fig. 2E). Moreover, the activity of SOD in the liver was found to be significantly increased in riboflavin-treated mice (12.35 ± 2.20) (P < 0.05) (Fig. 2D). The GSH concentration in the hepatic parenchyma was found to be preserved in SHAM animals (0.262 ± 0.005), but it reduced significantly following liver reperfusion in the I/R group (0.065 ± 0.008) (P < 0.01). However, the hepatocellular reserves of GSH were partially recovered by riboflavin administration (0.136 ± 0.011) (P < 0.05) (Fig. 2E). Then, we assessed the hepatic content of NOS by Western blotting, and it was found that riboflavin also reduced the levels of both eNOS and iNOS in the livers subjected to I/R and treated with riboflavin when compared with those treated with saline (Fig. 2L).

4. Discussion

Interruption of the blood supply to the liver and the subsequent reperfusion leads to an intense acute inflammatory response and exacerbated oxidative stress, which may cause significant liver injury and dysfunction. In the present work, we investigated the protective potential of riboflavin on liver I/R injury in mice. Riboflavin markedly decreased hepatocellular injury following normothermic I/R, as demonstrated by reductions in both the parenchymal damage score and the serum AST and ALT levels when compared with saline-treated animals. In agreement with these findings, some studies have also reported the attenuation of I/R injury in other organs, including the heart (Iwanaga et al., 2007; Mack et al., 1995; Kotelga et al., 1994), brain (Betz et al., 1994), and lungs (Seekamp et al., 1999), following riboflavin administration.

In accordance with the increase in the degree of hepatocellular damage after I/R, riboflavin reduced immunostaining for the 4-HNE adduct and nitrotyrosine in liver, both of which are well-known markers of tissue injury mediated by ROS and reactive nitrogen species (RNS). These results indicate that riboflavin suppressed oxidative/nitrosative stress in the setting of liver I/R. In a murine heterotopic cardiac transplantation model, riboflavin also reduced myocardial oxidative stress following hypothermic I/R (Iwanaga et al., 2007).
The antioxidant potential of riboflavin has been attributed to its active metabolite, dihydroriboflavin, which is produced by the action of the NADPH-dependent flavin reductase on riboflavin. Dihydroriboflavin plays a critical antioxidant role, as it rapidly reduces the Fe(IV)O and Fe(V)O oxidation states of heme proteins in vitro (Xu and Hultquist, 1991), states that have been implicated in tissue oxidative damage following I/R in many organs (Hultquist et al., 1993). Using Northern blot analysis, Mack et al. (1995) demonstrated NADPH-dependent flavin reductase gene expression in the heart, liver, lungs and kidneys, with much higher flavin reductase expression found in the liver. This finding allows us to theorise that the systemic infusion of high concentrations of riboflavin, as in
the current study, may result in the elevated production of dihydroriboflavin intracellularly and greater antioxidant activity in the liver parenchyma.

Oxidative stress has been described as a disturbance in the equilibrium status of ROS generation and the cellular antioxidant defence system. GSH is considered the major endogenous antioxidant produced by hepatocytes, participating directly in scavenging free radicals and ROS as well as maintaining exogenous antioxidants in their reduced (active) forms (Scholz et al., 1997; Altas et al., 2011). Riboflavin deficiency may decrease glutathione

Fig. 2. Representative photomicrographs of the SHAM, I/R, and I/R riboflavin groups. Liver sections were processed for 4-hydroxynonenal (4-HNE – magnification: 100×) (A–C) or nitrotyrosine immunostaining (magnification: 100×) (G–I). Effect of I/R or I/R plus riboflavin treatment on 4-HNE immunoreactivity (D), on the tissue content of reduced glutathione (GSH) (E), on hepatic superoxide dismutase (SOD) activity (F), on nitrotyrosine immunoreactivity (J), on the hepatic nitric oxide (NO) level (K), and on liver endothelial and inducible nitric oxide synthase (eNOS/iNOS) and β-actin expression (L). The data are reported as mean ± S.E.M. ● P < 0.01 I/R × SHAM; ○ P < 0.05 I/R × I/R riboflavin.
reductase activity, which mediates the regeneration of cellular stores of GSH, culminating in oxidative damage to proteins and DNA in hepatocytes (Manthey et al., 2006). FAD, the most important biologically active form of riboflavin, acts as a coenzyme for glutathione reductase (Powers, 2003). In agreement with these reports, our results showed that riboflavin partially restored the liver reserves of GSH in mice subjected to hepatic I/R. In addition, riboflavin significantly increased the activity of SOD in the liver following normothermic I/R, showing its protective role of hepatocellular antioxidant capacity.

Among the potential mechanisms contributing to hepatic I/R injury, neutrophil transmigration through the sinusoidal endothelium has been recognised as a pivotal factor. After transmigration, PMNs produce and release ROS, which can cause direct cellular damage and/or act as second messengers in signal transduction pathways involved in the regulation of genes encoding proinflammatory cytokines (Jaeschke and Smith, 1997; Jaeschke, 2006). In the present study, the number of liver-infiltrating neutrophils was remarkably diminished by riboflavin when assessed by both the MPO activity assay and counting PMNs in hepatic histological preparations. These findings suggest that the riboflavin-induced attenuation of hepatic oxidative stress may also be directly related to the reduction in neutrophil recruitment into the liver.

Consistent with these findings, previous studies have also reported powerful anti-inflammatory properties of riboflavin. The proteasome is a key regulator of inflammation, as it modulates the induction of inflammatory mediators such as tumour necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and NO in response to a variety of stimuli (Qureshi et al., 2003, 2005). Recently, Qureshi et al. (2011) demonstrated that riboflavin acted as a potent inhibitor of the chymotrypsin-like, trypsin-like and post-glutamase activities of the proteasomes in RAW 264.7 murine macrophages and of the chymotrypsin-like activity of 20S proteasomes in rabbit muscle tissue. They also demonstrated that riboflavin suppressed lipopolysaccharide (LPS)-stimulated TNF-α secretion, NO production, degradation of P-I and activation of NF-κB in RAW 264.7 cells and down-regulated TNF-α and iNOS gene expression in thioglycolate-elicited murine peritoneal macrophages. In addition, utilising an experimental model of endotoxin-induced sepsis and Gram-negative and Gram-positive bacterial infection, Toyosawa et al. (2004) revealed that riboflavin down-regulated serum levels of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, interferon-γ and macrophage inflammatory protein-2, as well as NO, resulting in a substantial increase in the survival rate following murine septic shock. Similarly, in a model of murine sepsis induced by a lethal LPS challenge, riboflavin was shown to reduce the plasma and tissue concentrations of IL-6 and MIP-2 as well as plasma NO levels and liver iNOS expression (Kodama et al., 2005). In agreement with these authors, our present results demonstrated that riboflavin could decrease hepatic eNOS and iNOS expression and modulate liver NO levels in the setting of normothermic liver I/R in mice.

5. Conclusion

In conclusion, the results of the current study provide evidence indicating that riboflavin exerts potent antioxidant and anti-inflammatory effects in the ischaemic liver, protecting hepatic parenchymal cells against I/R injury. The mechanism underlying these effects appears to be related to the intrinsic antioxidant potential of riboflavin/dihydroriboflavin and to the reduction in hepatic eNOS and iNOS expression and NO levels, culminating in the attenuation of oxidative/nitrosative stress and the inflammatory response following normothermic I/R in the liver. Considering the numerous beneficial effects of riboflavin demonstrated by us and other authors and that riboflavin is relatively inexpensive and essentially nontoxic, clinical trials should evaluate its potential for the treatment of inflammatory diseases and/or disorders involving acute oxidative damage.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

This research was supported by CNPq (fellowship to Sheila C. Sanches no. 160028/2012-4), FAEPESP (project Grant no. 2008/10296-2) and FAEP.

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Moloney, W.C., Mcpherson, K., Fliegelman, L., 1960. Esterase activity in leukocytes and liver iNOS expression (Kodama et al., 2005). In agreement with these authors, our present results demonstrated that riboflavin could decrease hepatic eNOS and iNOS expression and modulate liver NO levels in the setting of normothermic liver I/R in mice.