**HOSPITAL OF CLINICS FROM PORTO ALEGRE**

**Research and Graduate Group**

**Scientific Committee and Committee on Ethics in the Use of Animals**

**Research Project Opinion**

**Project**

**2010-0316** - Melatonin protects the liver from carbon tetrachloride-induced damage in rats - Study of liver fibrosis

**Responsible Researcher:** NORMA ANAIR POSSA MARRONI

**Origin:** HCPA >> Serviço de Centro de Pesquisa Experimental

**Realization:** HCPA >> Unidade de Experimentação Animal

**Structured Summary - Introduction:**

Liver diseases are one of the biggest health problems in the world. In this context, fibrosis liver disease and cirrhosis represent the most common pathological manifestations and are among the largest causes of human mortality (Elsharkawy, Oakley et al. 2005; Lotersztajn, Julien et al. 2005; Schuppan and Afdhal 2008). Cirrhosis is characterized by the formation of nodules, anatomical changes and changes in liver function due to the death of hepatocytes. Such structural changes constitute the main tissue responses liver to numerous aggressions of an inflammatory, viral, toxic, metabolic or congestive nature (Friedman 2003; Heidelbaugh and Sherbondy 2006; Tsukada, Parsons et al. 2006). Due to the great worldwide problem that cirrhosis represents, many researches about this disease are carried out all over the world, aiming to test substances and techniques that can be converted into treatment and search for a cure, or at least increase the patient's survival, preventing the progression of disease. Ethical considerations limit procedures in human beings, reinforcing the need for models animals that reproduce the pathological picture of cirrhosis (Laleman, Vander Elst et al. 2006). The experimental model of liver cirrhosis by the administration of carbon tetrachloride (CCl4) is widely used for the study of the development of the pathological process and for research treatment alternatives. In this model, liver fibrosis and regenerative nodules are prominent and the Histological pattern is similar to human cirrhosis (Cameron 1936; Perez Tamayo 1983; Jimenez, Claria et al. al. 1992; Cremonese, Pereira-Filho et al. 2001; Pereira-Filho, Ferreira et al. 2008). The mechanism of action of CCl4 involves its metabolism by cytochrome P450, which stimulates the production of free radicals (RLs). These cause necrosis of hepatocytes, induce inflammation and promote greater progression of fibrosis (Basu 2003). There is growing evidence that changes in redox homeostasis may play a role. Significant in the pathogenesis of many diseases characterized by chronic inflammation, process activation of healing and fibrogenesis of hepatic tissue (Novo and Parola 2008). experimental studies and studies clinics have shown that lipoperoxidation (LPO) is often associated with the development of liver fibrosis (Tsukada, Parsons et al. 2006; Fang and Lin 2008). Given the importance and growing prevalence of the subject, our study aims to analyze the complications of cirrhosis, the involvement of oxidative stress, seeking to understand molecular mechanisms involved, in an attempt to elucidate the effect of melatonin acting as antioxidant in an attempt to prevent or slow the progression of fibrosis, reducing the action of radicals free of oxygen and nitrogen related to

oxidative and nitrosative stress, causing damage hepatic. Melatonin may become, in the future, a valuable therapeutic element, increasing the survival of cirrhotic patients until the time of transplantation.

**Structured Summary - Methodology:**
This study has a comparative experimental character, where the induction of cirrhosis in rats will be performed Wistar males, through the inhalation of carbon tetrachloride, comparing them with control and treated with melatonin. Forty animals will be used, with an average weight of 250g, divided into 4 groups: Control (CO), melatonin (MEL), carbon tetrachloride (CCl4) and melatonin carbon tetrachloride (CCl4+MEL). The rats will be subjected to CCl4 inhalations (2x/week) for 16 weeks, receiving phenobarbital in drinking water at a dose of 0.3g/dl, as an enzyme inducer. Melatonin (20mg/Kg i.p.) started in the 10th week of inhalation, lasting until the end of the experiment. Liver function will be assessed through the enzymes, aspartate aminotrasferase (AST), alanine aminotransferase (ALT) and albumin, by automated method. To verify the presence of hepatic collagen, analyzes will be performed histologics where the slides will be stained with picrosirius and by measuring the content of hydroxyproline. The activation of stellate cells will be verified through a-SMA immunohistochemistry. Protein expression of collagen type I alpha 2, collagen type III alpha 1, MMP-2, TIMP1, TGF-b1, SMAD2\3 and PDFGb in liver tissue will also be evaluated by western blot. Lipoperoxidation will be evaluated through the method of reactive substances to thiobarbituric acid (TBARS). The Antioxidant Response cell will be evaluated through the expression of nuclear transcription factor 2 (Nrf2) and its association with Keap1 protein through immunohistochemistry, as well as evaluating the expression of enzymes by western blot antioxidants (SOD, CAT and GPx). Also to assess hepatic oxidative damage, the reduced glutathione / oxidized glutathione (GSH/GSSG) ratio. To assess the DNA damage, the bone marrow micronucleus test and the comet assay in blood and liver tissue. The sample size calculation is based on our previous study, in which to detect differences with magnitude (effect size: I/O) equal to 2.0 standard deviation units in the oxidative balance, keeping a = 0.05 and power of 90%, ten (10) rats per group were calculated. The generated quantitative data will be described by mean and standard deviation. In the presence of asymmetry it will be performed logarithmic transformation of them. The comparison of groups will be performed by the procedure of analysis of repeated measures variables or according to the experimental model by ONEWAY ANOVA. In finding the difference will be performed by Tukey's procedure and the data analyzed with the SPSS program.

**Structured Summary - Objectives:**
Given the importance and growing prevalence of the subject, our study aims to analyze the complications of cirrhosis, the involvement of oxidative stress, seeking to understand mechanisms involved, in an attempt to elucidate the effect of melatonin acting as an antioxidant in attempt to prevent or slow the progression of fibrosis by reducing the action of oxygen free radicals and nitrogen related to oxidative and nitrosative stress, causing liver damage. Melatonin it may become, in the future, a valuable therapeutic element, increasing the survival of patients cirrhotics until the time of transplantation. General This work aims to evaluate the effects of melatonin in the modulation of oxidative balance and in the fibrogenic process of liver disease, using an experimental model of inhaled CCl4-induced cirrhosis in male Wistar rats. Specific 1) Evaluate liver integrity through enzymes, aspartate aminotrasferase (AST), alanine aminotransferase (ALT) and albumin; 2) Carry out anatomopathological test on the liver tissue, through the hematoxylin and eosin and picrosirius staining; 3) Quantification of hepatic collagen, by measuring the hydroxyproline content; 4) Assess a-SMA immunoreactivity in liver tissue by immunohistochemistry. 5) Check the protein expression of collagen type I alpha 2, collagen type III alpha 1, MMP-2, TIMP-1, TGF-b1, SMAD2\3 and PDFGb in liver tissue. 6) Assess lipoperoxidation, through the method thiobarbituric acid reactive substances (TBARS); 7) Check the expression of Nfr2 and Keap1 by immunohistochemistry. 8) Verify the protein expression of antioxidant enzymes (SOD, CAT and GPx). 9) Quantify the liver activity of GSH and GSSG, as well as verify the GSH/GSSG ratio. 10) Evaluate DNA damage in the liver cirrhosis model

and the action of melatonin using the micronucleus test in bone marrow and comet assay in blood and liver tissue.

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**General comments about the project:**

Once the pending issues were met and as the support areas (UEA and UPE) have already given their opinions, the budget was approved by FIPE in the amount of R$10,627.90.

**Date of Delivery of Opinion:** 07/05/2011 18:56:47

**Decision:** Approved