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NOTE

Menhaden Oil Rich Diet and Experimental Renal Damage Due to Ischemia Reperfusion

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Abstract: Renal necrosis can be induced in weanling rats due to choline deficient diet. Menhaden oil has a protective effect against the development of renal necrosis in choline deficient weanling rats. The aim of this work was to determine the effects of menhaden oil in a model of acute kidney injury due to ischemia reperfusion. Wistar rats were divided into two groups and fed vegetable oils or menhaden oil as lipids. Unilateral renal ischemia was performed for 30 minutes and animals were sacrificed 48 hours later. Histopathological examination showed no significant differences between groups. Menhaden oil did not prevent histopathological lesions.

Key words: menhaden oil, acute kidney injury, ischemia reperfusion, histopathological damage

1 INTRODUCTION

Tubular focal necrosis, which may extend to massive cortical necrosis, developing acute renal failure can be experimentally induced in weanling rats due to a choline deficient (CD) diet[1]. Menhaden fish oil has a high protective effect against the development of renal necrosis when given in the diet as a source of lipids in CD weanling rats[2]. Menhaden oil is a very important source of omega-3 fatty acids.

In humans there are diverse causes of acute kidney injury, such as renal ischemia[3]. In some cases patients who develop this syndrome do not recover full renal function, developing end-stage renal failure; requiring either, dialysis or kidney transplant[4]. Further, it is very important the development of new models of acute kidney injury in order to understand the mechanisms underlying the progression of kidney diseases[5]. The aim of this work is to determine the possible histopathological effects of menhaden oil in a model of acute kidney injury due to ischemia reperfusion (IR).

2 EXPERIMENTAL

Fourteen adult male Wistar rats (200 ± 20 g) from the Center for Experimental and Applied Pathology were divided into two groups (n = 7) and fed for two weeks the following diets: (1) diet with vegetable oils as lipids [20 g/100 g of balanced diet; 14 g of hydrogenated vegetable oil (Vegetalina Dánica, Buenos Aires, Argentina) and 5.7g of corn oil (Mazola, Córdoba, Argentina)] and (2) diet with menhaden oil as lipid [20 g/100g of balanced diet (MPB 296012 , Solon, Ohio, USA)]. Diets were kept at 4℃ away from the light and were replaced every day. Authors have adhered to appropriate NIH Guide for the Care and Use of Laboratory Animals. This protocol was approved by the Animal Care and Use Committee of the School of Medicine, University of Buenos Aires. After fourteen days, the animals were anesthetized with ketamine/xylazine (50/10 mg/kg body weight). Abdomen was opened through a lateral incision and the left renal pedicle was exposed. A microvascular clump was placed in the left renal pedicle to completely block renal blood flow. Thirty minutes later, the clump was removed allowing renal reperfusion. After 48 hours, the animals were sacrificed and arterial blood samples were collected from the abdominal aorta. The left kidney was removed, fixed in buffered formalin and em-

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bedded in paraffin. The sections were cut and stained with hematoxylin/eosin to analyze the existence of histopathological alterations.

3 RESULTS

Histopathology: According to Montes de Oca *et al.*[1], the histopathological classification of renal necrosis was divided into 5 grades: no necrosis (grade 0), necrosis involving less than 25% of the organ (grade 1), between 25 and 50% (grade 2), between 50 and 75% (grade 3) and between 75 and 100% (grade 4). Animals fed diet 1 showed: grade 1 injury (n = 2), grade 2 (n = 1), grade 2-3 (n = 1), grade 3-4 (n = 2) and grade 4 (n = 1) (Fig. 1). Rats fed diet 2 showed: grade 0 (n = 1), grade 0-1 (n = 1), grade 1-2 (n = 1), grade 2-3 (n = 1), grade 3-4 (n = 1) and grade 4 (n = 2) (Fig. 2). Necrosis, characterized by pyknosis, cariolysis and increased eosinophilia, involves tubules and glomeruli, mainly in the junction area between cortex and medulla. These results show no significant differences between both groups.

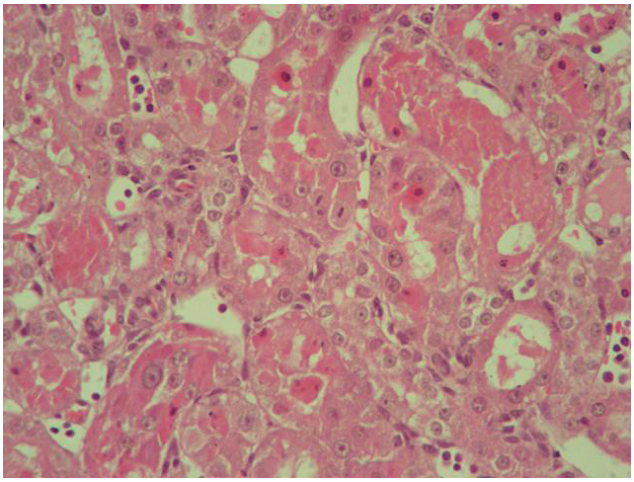

Fig. 1

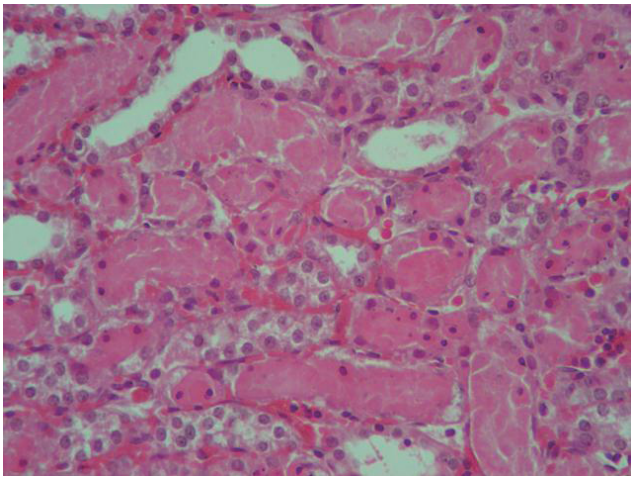

Fig. 2

4 DISCUSSION

The protective effects of renal damage through omega-3 menhaden fish oil and its main compounds, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been widely demonstrated[2]. However, there are still controversial areas. One of them is presented here, in the renal IR model. Previously, our laboratory demonstrated that only in weanling rats renal cortical necrosis with acute renal failure could be induced by a diet deficient in methyl donors, such as CD[1]. Subsequently, in the same model, menhaden oil administration significantly protected against kidney damage[2] at least in part by reducing oxidative stress (OS)[6].

Ashtiyani *et al.*[7] found that omega-3 administration decreased renal histopathological damage in rats submitted to an IR model. The results presented here show no significant changes in renal histopathological examination in rats with IR-induced renal necrosis that were fed menhaden oil in the diet (Fig. 2) compared to the untreated ones (Fig. 1). Although renal injury was similar, a final stage with cortical necrosis; decrease and/or prevention was not achieved. It could be considered that, in addition to the age and rat strain, the route of administration and the dose were different.

Ajami *et al.*[8] concluded that pre-ischemic exposure to DHA + EPA in IR could improve the outcome of early graft function and cell death by inhibition of IR-induced oxidative stress. In the reports of Ashtiyami and Ajami, DHA and EPA were given by gavage, while in our experiment menhaden oil was administered with the diet. As for the dose and route, it is interesting to note that Kielar *et al.*[9] conclude that DHA improves ischemic acute renal failure (ARF) in mice and also inhibits inflammatory molecules associated with ARF, such as TNF and iNOS. In that experiment the intraperitoneal route was used and the response to DHA was not dose dependent. In addition, it could be speculated that, although there is a final common stage, the pathophysiology of these four experiments (Ashtiyani, Ajani, Kielar and the present) might not be.

Prevention of kidney damage on CD weaning rats also involves other organs such as the liver. Also, the elapsed time in the treatment was much longer on CD, 10 days versus two days in IR. In terms of time, it is important to note that the CD model works in weanling rats while adult rats were used here. In addition, IR has other organs involved only in the final stage. Another issue to consider is OS. It is accepted that the mitochondrial complex syndrome I triggers OS and it is a pathophysiological pathway shared by CD and IR. Furthermore, in both of them, CD

and IR, menhaden oil, DHA alone or plus EPA, decrease or prevent the occurrence of OS.

The age, route of administration, dose and treatment time could be considered as a possible explanation of the different results. In addition, another question to answer is what is it the difference in the pathophysiology of CD and IR. One possible answer is that the peroxidation of phospholipids due to OS in IR is mainly renal and in CD it extends concomitantly to other organs such as liver, brain and heart.

5 CONCLUSION

Under these experimental conditions, menhaden oil does not prevent or decrease the histopathological lesions. This result is in disagreement with Ashtiyani 2012 and Ajami 2012 and it could be suggested that differences in age, route, dose, treatment time and pathophysiologic pathways may support the present results.

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