Arthrospira maxima prevents the acute fatty liver induced by the administration of simvastatin, ethanol and a hypercholesterolemic diet to mice

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Abstract

An evident fatty liver, corroborated morphologically and chemically, was produced in CD-1 mice after five daily doses of simvastatin 75 mg/Kg body weight, a hypercholesterolemic diet and 20 percent ethanol in the drinking water. After treating the animals, they presented serum triacylglycerols levels five times higher than the control mice, total lipids, cholesterol and triacylglycerols in the liver were 2, 2 and 1.5 times higher, respectively, than in control animals. When \textit{Arthrospira maxima} was given with diet two weeks prior the onset of fatty liver induction, there was a decrement of liver total lipids (40%), liver triacylglycerols (50%) and serum triacylglycerols (50%) compared to the animals with the same treatment but without \textit{Arthrospira maxima}. In addition to the mentioned protective effect, the administration of this algae, produced a significant increase (45%) in serum high density lipoproteins.

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The mechanism for this protective effect was not established in these experiments. © 2002 Elsevier Science Inc. All rights reserved.

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**Introduction**

Previous studies report that high vastatin (VTS) doses combined with a hypercholesterolemic diet, result in great toxicity for CD-1 male mice [1,2]. These toxic effects are manifested by an increase in liver mass as well as the accumulation of triacylglycerols in the liver, compared to the normal control group. On the other hand, the protective effect of *Arthrospira maxima* (*Spirulina maxima*; AM) on fatty liver production, has been previously demonstrated in rats with high fructose diet [3], carbon tetrachloride injection [4] or in mice with alloxan-induced diabetes [5]. In this report the induction of an acute fatty liver in mice was achieved after five daily doses of simvastatin (SVT) 75 mg/Kg, combined with a hypercholesterolemic diet and ethanol in the drinking water. Using this acute fatty-liver induction the preventive effect of AM was tested.

**Methods**

CD-1 male mice weighing 25-30 g were used. Three groups of 10 mice each were formed as follows: control mice (CM), hypercholesterolemic treatment (HT), and HT plus *Arthrospira maxima* (HT+AM). CM group received a commercial Purina Chow with 27% protein, 5% fat content along the entire experiment. The HT group received during two weeks the control diet and then five days a diet containing 1% cholesterol and 0.1% sodium deoxycholate. The HT+AM group received during two weeks a control diet supplemented with 10% AM and five days the hypercholesterolemic diet + AM. To complete the treatment, groups HT and HT+AM received along with the hypercholesterolemic diet a daily dose of 75 mg/Kg body weight of simvastatin in a volume of 0.2 ml of 0.1% aqueous methyl cellulose administered by gavage, and 20% ethanol in the drinking water. Simvastatin was a donation from Merck Sharp and Dhome de México SA de CV; *Arthrospira maxima* was a donation of Spirulina Mexicana SA de CV (México). Cholesterol and bile acids were obtained from Sigma Chemical. Following the five-day treatment the animals were starved 12 h before being decapitated and blood was collected to determine cholesterol, triacylglycerols (TAG) and lipoproteins. Enzymatic commercial kits (Roche SA de CV), were used for these analyses. Lipoprotein fractions were separated by electrophoresis and their cholesterol content was determined using an enzymatic method in agarose gel from Ciba-Corning [6].

Liver was excised and the total lipid content was evaluated by a modified Folch’s method [7]. Cholesterol and TAGs were determined in the lipid extract as mentioned above. Livers
were also processed for microscopic studies by routine staining with hematoxylin and eosin of the tissue sections. The results were analyzed by the ANOVA statistics with a \( p<0.05 \) (*), a \( p<0.01 \) (**), or a \( p<0.001 \) (***) significance.

**Results**

Fig. 1 shows that the hyperlipidemic treatment given during the last 5 days of the experiment produced a lower body weight of HT and HT+AM mice compared to the values of the control group, the weight loss being smaller in the group HT+AM, than in the group HT (without AM) \( (p<0.05) \). On the other hand, liver mass increased in both HT and HT+AM groups, being this value smaller in the animals with AM included, but the difference was not statistically significant (Fig. 1).

Total lipids, cholesterol, and TAG in the liver, and cholesterol and TAG in serum are shown in Fig. 2. The HT group showed the highest values, but the administration of AM prevented the increase in liver total lipids \( (p<0.05) \), liver TAG \( (p<0.001) \) and serum cholesterol \( (p<0.001) \), but not in liver cholesterol or serum TAG. Fig. 2 also shows the liver thiobarbituric acid

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**Fig. 1.** *Arthrospira maxima* effects on body weight and liver mass (inset) through the experimental time. Results are expressed as the mean±SEM; \( n=10 \), CM=control mice, HT=hypercholesterolemic treatment, HT+AM=hypercholesterolemic treatment + *Arthrospira maxima*. Body weight in HT vs. HT+AM, \( p<0.05 \).
Fig. 2. *Arthrospira maxima* effects on liver and blood lipids, and liver malonaldehyde MDA, TBARS. Results are expressed as mean±SEM. CM=control mice, HT=hypercholesterolemic treatment, HT+AM=hypercholesterolemic treatment + *Arthrospira maxima*; n=10. TL=total lipids, TAG=triacylglycerols, CHOL=cholesterol. In HT vs. HT+AM: liver TL p<0.05, liver TAG p<0.001; serum CHOL p<0.001.
reactant substances (TBARS), expressed as nmol/g malondialdehyde (MDA). The hyperlipidemic treatment increased significantly the MDA values but again the administration of AM prevented this increase, however the differences were not statistically significant.

The HT group presented an important decrease in HDL-cholesterol concentration. This effect was reversed by the inclusion of AM in the diet. The serum lipoprotein profile was evaluated by electrophoresis followed by the enzymatic determination of cholesterol in HDL. HDL (filled bar) and non-HDL (open bar) fractions are expressed as percentage of total cholesterol (Fig. 3).

Fig. 3. *Arthrospira maxima* effects on serum lipoproteins. Results are expressed as mean±SEM, n=10. CM=control mice, HT=hypercholesterolemic treatment, HT+AM=hypercholesterolemic treatment + *Arthrospira maxima*. Results are expressed as percentage of total cholesterol in lipoprotein fractions. HDL=high density lipoproteins, NON-HDL=non-HDL (low density lipoproteins + very low density lipoproteins). For HT vs. HT+AM: HDL% p<0.01, NON-HDL% p<0.01.

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Fig. 4. Representative macroscopic and microscopic images of the liver, in the later case after hematoxylin-eosin staining. This mouse received simvastatin 75 mg/Kg/day, a hypercholesterolemic diet and 20% ethanol in the drinking water (HT group) during 5 days.
Fig. 4 includes the macroscopic and microscopic images of the liver in an animal from the HT group. The microscopic study shows a toxic hepatitis characterized by inflammatory infiltration, necrosis and vesicular steatosis.

Discussion

In order to obtain an acute fatty liver, we proved SVT daily doses 30–500 mg/Kg body weight in mice fed with normal Purina Chow diet or a hypercholesterolemic diet during 10 days. The combined administration of SVT with control diet did not alter liver mass, total lipids, and TAG (data not shown). However, when a hypercholesterolemic diet was administered simultaneously with SVT, at least 62 mg/Kg body weight during 10 days, it was enough to induce biochemical and pathological modifications. A SVT daily dose of 125 mg/Kg body weight, caused the death of most animals in a week. This synergistic activity was previously reported [2]. It has been suggested that exogenous cholesterol inhibits the up-regulating expression of mRNA for 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMG Co-A reductase) [8,9], that appears after the intracellular mevalonate deficit in the liver. The inhibition of HMG-CoA reductase produces a depletion of the mevalonate metabolites which are critical for cellular viability [10]. Furthermore, it has been demonstrated that statins affect the synchronization of the cellular division in the early G1 state, and this effect is increased by cholesterol. The inhibitory action of statins on cellular proliferation has also been investigated in tumoral cells [11,12].

The statin utilized in this work (simvastatin) is a hydrophobic drug with the capacity to induce apoptosis on cultured vascular smooth muscle cells in the rat. The apoptotic effect can be reversed by the addition of mevalonate, farnesyl pyrophosphate or geranylgeranyl pyrophosphate [13].

Due to the synergistic action of high vastatin doses combined with a hypercholesterolemic diet, this procedure has been proposed to be adequate to evaluate comparatively in vivo the toxicity of vastatins [1]. The SVT dose used in this work is almost 100 times higher than that recommended for a 50 Kg person (40 mg/day), to inhibit cholesterol synthesis. Similar doses have been employed to inhibit tumoral growth. High vastatins doses have also shown toxicity and induce apoptosis in cell cultures [14].

The effect of AM supplementation on body weight is fully explained by the nutritional quality of this algae. Just before the application of SVT treatment during the last five days of the experiment, the group that received 10% AM in the diet presented higher body weight than the animals without AM (p<0.05). On the other hand, when AM administration was initiated two weeks before the onset of fatty liver induction it produced a decrease of liver total lipids, the effect being mainly on TAG. However, if AM administration was initiated simultaneously with the fatty liver induction, it was observed a poor effect on hepatic and serum lipids (results not shown). These results can be partially explained due to the fact that certain antioxidant components of AM (tocopherols, phenolic acids, phycocyanin, selenium, beta-carotene) may be stored in the liver and were not sufficiently available when AM was not given prior to the fatty liver induction.
The protective effect of AM on liver TAG, and serum cholesterol, is in accordance with the results obtained with AM in rats which received carbon tetrachloride or a high fructose diet as fatty liver inducers [3,4]. The hypocholesterolemic effect of AM on mice agrees with other reports when it was given (16%) in a normal diet [15], with a high-fructose diet [16], or when AM was administered to humans, 4.2 g/day during 4 weeks [17].

Because of the great relevance of the association of high cholesterol serum levels and premature atherosclerosis [18,19], further investigation about the hypocholesterolemic action of AM in humans is necessary, specially if AM favors the cholesterol-reverse transport and its elimination.

The decreased TBARS levels produced by AM in the liver were not statistical significant. A similar finding was observed when lovastatin, 67 mg/kg body weight, and 20% ethanol in the drinking water, were given to rats during five weeks [20]; however, in those experiments it was found a decreased values of the liver Coenzyme Q. On the other hand, when carbon tetrachloride was used to induce fatty liver in rats, AM decreased significantly the liver TBARS [4]. It is well known that hepatic toxicity of CCl₄ is expressed as an oxidative stress through the action of trichloromethyl (CCl₃/C1) and C1 [21]. In addition, there are reports on the in vivo and in vitro antioxidant effects of AM [22].

Furthermore, it has been reported that AM induces body weight loss in obese subjects [23] as well as a decrease in total cholesterol plasmatic levels [17], it also decreases cancer risk [24–26], improves malnutrition states, favors the immune response [27] and the antimicrobial activity. Spirulina platensis inhibits HIV 1 replication [28] and enhances humoral-and cell-mediated immune functions [29].

The 20% ethanol in the drinking water was used in this report to diminish the elimination of SVT, since SVT and ethanol could be competing for the same removable mechanism [30]. Moreover, the ethanol levels used in this report could mimic the amount consumed by a human being.

According to the beneficial effects shown by AM preventing fatty liver, it is important to do further studies to estimate the potential application of these effects on patients with lipid disorders.

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