Hepatoprotective Effect Of Ethilendiaminotetracetic Acid And Sodium Selenate In A Chronic Hepatic Failure Model In Mice

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Citation

J Granados, P Sobenes, J Astacio, M Bertoli. *Hepatoprotective Effect Of Ethilendiaminotetracetic Acid And Sodium Selenate In A Chronic Hepatic Failure Model In Mice*. The Internet Journal of Gastroenterology. 2012 Volume 11 Number 1.

Abstract

Oxidative stress and serum iron levels are important in the development of hepatic fibrosis and low serum selenium levels in some hepatic diseases. The objective was to determine the hepatoprotector effect of EDTA (ethilendiaminotetracetic acid) as a chelating agent and SeNa(sodium selenate) in a chronic hepatic failure model in mice. Iron chelating therapy with deferoxamine is more expensive than EDTA representing an economic alternative. This is an experimental controlled trial with heterocigotes adult mice grouped in 4 experimental groups. Group I received no treatment, groups II and III each treatment separately, and group IV combined treatment. Group I revealed chronic hepatic injury ($p<0.05$); groups II and III revealed similar findings; however initial low damage was observed in 60% with turbid and vacuolar degeneration ($p<0.05$). Group IV revealed 25-75% of the subjects without histopathology degeneration and 50-100% did not present necrosis ($p<0.01$). This was the only group with normal enzymatic values obtaining 65.3% of hepatic protection. Conclusion: using a chronic hepatic failure model with thioacetamide, the combination of EDTA and SeNa produce a better hepatoprotection response in contrast with monotherapy group in the reduction of the progression and severity of hepatic damage.

ABBREVIATIONS

INTRODUCTION

Chronic hepatic diseases, are common in developing countries. Chronic hepatic diseases in El Salvador are the fourth cause of morbidity (according to Public Health Ministry data of 2008)(2). Oxidative Stress (OS) and serum iron levels have demonstrated an important role in the development and progression of hepatic fibrosis(1) and low serum selenium levels have also been found in some hepatic diseases(3) and the sodium selenate availability is low in volcanic tropical soils, such as Central American countries(14)(15). Iron depletion impedes OS, inflammation and Hepatic Stellate Cells (HSC) activation, producing an inhibition of hepatic fibrosis.(1) Selenium is also important for oxidative lipids reduction,(10) and its deficiency has been related to several pathologies.(11) Therefore the control of the homeostasis of the hepatic iron can be a therapeutic strategy in chronic hepatic diseases.(1)

The aim of this study was to determine the hepatoprotector effect of Ethilendiaminotetracetic acid (EDTA) as a chelating agent and Sodium selenate (SeNa) as an antioxidant agent; hypothesizing that both therapies diminish chronic hepatic failure progression in a mice model. The importance of this combined therapy for the reduction of oxidative stress has not been defined at this time. Deferoxamine as a chelating agent specific for the iron in HSC. However deferoxamine is more expensive than EDTA therapy which represents an economic alternative. The study consisted in an experimental controlled trial using heterocigotes female adult mice grouped in 4 experimental groups. Group I received no treatment, groups II and III each treatment separately, and group IV combined treatment. Microscopically group I revealed histopathological findings equal to chronic hepatic injury ($p<0.05$); groups II and III revealed similar findings, however initial low damage was observed in 60% of tissue samples with turbid and vacuolar degeneration ($p<0.05$). Group IV revealed 25 -75% of the subjects without histopathology degeneration and 50-100% did not present necrosis ($p<0.01$). This was the only group with normal enzymatic values obtaining a 65.3% of hepatic protection. In conclusion: using an experimental chronic liver failure model with thioacetamide, the combination of EDTA and SeNa produce a better hepatoprotection response (65.3%) in contrast with monotherapy group in the reduction of the progression and severity of hepatic damage.

METHODS

The study is an experimental controlled trial, with adult female heterocigotes mice (more than 6 weeks old) from Swiss albino line. Breed in Dr. Jose Matias Delgado University installations with 12hr light and 12hrs dark ambience at 27°C temp using a KNINO diet (very similar to AIN-93G) and water ad libitum.

All animals were submitted to TAA during a period of 6 weeks for chronic hepatic injury induction (4)(12) due to its high specificity for the liver (5)(6) and the OS produced which contributes to lipid peroxidation and free radical production.(7)(8) EDTA and SeNa were applied separately and in combination to determine the reduction of hepatic damage and were distributed as shown in chart 1.

The N used was 20 mice divided in 4 groups. Each subject was randomly assigned to one of each group. Group 1=Control, group 2=EDTA, group 3=SeNa, group 4=EDTA+SeNa.

The experimentation protocol was based on Animal Welfare ACT (AWA) of the United States Department of Agriculture, causing minimal possible pain to mice in drug administration as in sacrifice methods with previous blood extraction for biochemical analysis; autopsy, and liver extraction for histopathological study as well.(17)

Figure 1

Chart 1 Experimental Design.

#	DESCRIPTION	INTERVENTION
I n=5	Chronic liver fibrosis using TAA	Intraperitoneal Injection of 200mg/kg of TAA twice a week
II n=5	SeNa Group	Intraperitoneal Injection of 200mg/kg of TAA twice a week + thrice a week administration of SeNa 500ug/kg with intragastric canule starting 2nd week
III n=5	EDTA Group	Intraperitoneal Injection of 200mg/kg of TAA twice a week +0.02mg/g of EDTA with Intraperitoneal injection twice a week starting 2nd week
IV n=5	EDTA+SeNa Group	Intraperitoneal Injection of 200mg/kg of TAA twice a week + thrice a week administration of SeNa 500ug/kg with intragastric canule starting 2nd week+ 0.02mg/g of EDTA with Intraperitoneal injection twice a week starting 2nd week

Administration times were selected accordingly to the half life of the compounds TAA 1.5hrs, EDTA 1hr and SeNa 40hrs. The dose and weeks of TAA administration were previously standardized in an animal (mice) model of chronic hepatic failure using TAA.

Given the variety of chronic hepatic damage markers, this study includes the most frequently used parameters.

Biochemical analysis was made using blood samples obtained by cardiac puncture. The following markers were measured to determine the extent of hepatic damage:

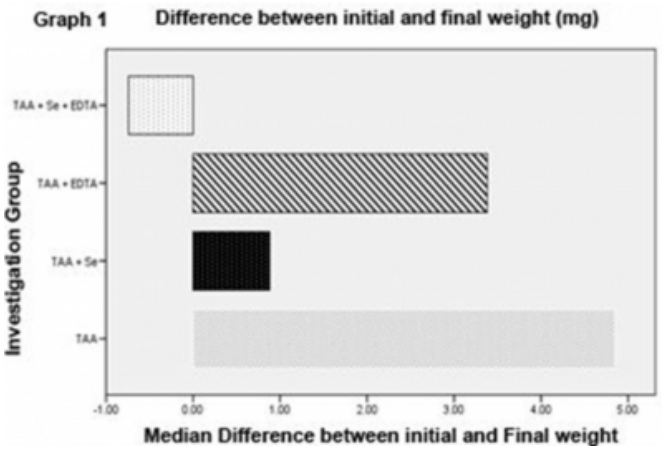
Data analysis was made with SPSS program version 15.0 using non-parametrical tests: U Man-Whitney for independent experiments and Kruskall-Walis.

RESULTS

The N of subjects was of 20 mice distributed as depicted on chart 1, with a final N of 19 mice since one of the test subjects in group IV died in the 4th week and was excluded of the experiment.

The initial to final bodyweight comparison of the subjects shows an increase in groups I, II and III (TAA, TAA+ SeNa, TAA+EDTA); however, the only group that revealed a decrease in bodyweight was group IV (TAA+EDTA+SeNa) as shown on graph 1.

Figure 2



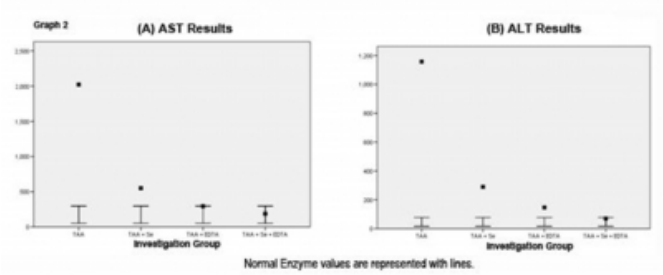
Macroscopically; dissection and extraction of the liver revealed groups I, II and III with pale and nodular hepatic surfaces, in contrast with group IV that showed shiny smooth surfaces although some were shiny nodular.

Histopathological findings in group I with no treatment, resulted in severe hepatic damage, revealing multifocal necrosis, hepatocyte degeneration in all subjects, and increased chronic hyperemia which precedes fibrosis formation, a known chronic marker in hepatic injury. Groups II and III, evidenced initial and mild hepatic damage. Meanwhile in group IV, 75% of subjects presented initial damages, and 25% without hepatic damages.

Biochemically; only in group IV, AST and ALT serum values are within normal range; as shown in the following

graph 2.

Figure 3



To evaluate the hepatic injury intensity, a score was established according to hepatic injury level, based on necrosis, hepatocyte degeneration and chronic hyperemia scales; obtaining average results for each group, as depicted on chart 3, with statistically significant difference ($p<0.05$)

DISCUSSION

There are different causes involved in chronic hepatic failure, activating a dynamic set of events; starting with hepatocyte damage, activation of inflammatory cells, OS, activation and proliferation of HSC.(7) OS plays a very important role in the induction of hepatic damage using TAA. (4)(5)(6) Owed also to the inhibition of Selenium biodisponibility in the liver, similar to alcoholic and non alcoholic hepatopathies. (7) Iron accumulation has been also described in some hepatopathies, increasing lipid peroxidation and contributing to initiation and perpetuation of hepatic injury.(9)

Total weight gain in groups as shown in graph 1, is consistent with systemic changes (liquid retention, hepatic weight increase) reported by Elinav.(12) The finding of weight reduction in the IV group suggests that the hepatic alteration caused by TAA did not influence the weight in this group.

Macroscopically, all liver surfaces, in group I, were pale, nodular and with thickened borders; compatible with expected hepatic damage. These finding were less prominent in groups II and III. However, in group IV the surfaces were smooth, shiny, and with normal sharp borders, a clearly contrast with group I; since all groups where exposed to hepatic injury with TAA.

Microscopically group I revealed findings equal to the chronic hepatic liver failure animal model using TAA. All animals presented turbid and vacuolar degeneration, multifocal necrosis from low to severe. In 40-60% chronic hyperemia was observed.

Both monotherapy groups (II and III) reveal similar findings to group I. In these groups (II,III) there were no severe damage present. Initial low damage was observed in 60% of tissue samples with turbid and vacuolar degeneration. Group III reveal more than 80% of samples without necrosis.

However combined therapy group reveal 25-75% of the subjects without histopathology degeneration; 50-100% did not present necrosis. Chronic passive hyperemia was present in low form in 75%. This data obtained in the experiment was statistically significant between groups I-IV. ($p<0.05$)

The intensity of hepatic damage was established based on a score as explained before, taking into account necrosis, hepathocytic degeneration, and chronic passive hyperemia; with unique exposure to TAA understood as a 0% protection and a 100% of no protection according to the intensity of the injury. Combined therapy group obtained the least hepatic damage intensity with a 65% hepatoprotection. (as shown in chart 3) This data is statistically significant for monotherapy and combined therapy groups compared to control ($p<0.05$).

Correlation between histopathology findings as the gold standard and transaminase values, indicates a significant association between EDTA + SeNa and lesion intensity. Indicating a decrease in histopathology progression of hepatic injury.

Figure 4

Chart 3.- Hepatic Intensity damage based on histopathology analysis.

	Investigation Group	N	Average Rate [a,b]	% Protection
Intensity	TAA	5	17.00	0
	TAA + SeNA	5	9.20	45.9
	TAA + EDTA	5	7.10	58.2
	TAA + SeNA + EDTA	4	5.88	65.3
	Total	19		

a. Statistically significant difference, Kruskal-Wallis Test
b Non Statistically significant difference. No significance between treatment groups, but present in contrast with control group (TAA) Dunn Test.

The extent of cellular death in monotherapy group II (TAA+SeNa) presents near normal enzyme values with a 72 -75% of hepatoprotection as shown in Chart 2. This improvement suggests the restitution of Selenium levels that are depleted from hepatic tissues using TAA according to studies. TAA+EDTA group obtained normal enzyme values with an 85-87.3% of hepatoprotection. The necrosis diminution in this group could be explained to the multiple effects of EDTA as chelating agent in cellular chemical reactions. EDTA is capable of chelating bivalents ions of

iron and other transition metals and intracellular messengers (calcium) avoiding free oxygen (FOR) and nitrogen radical (FNR) production in peroxidation reactions.(16)

Figure 5

Chart 2 – Hepatoprotection percentage according to serum enzyme concentrations

Enzymes	TAA	TAA+SeNa	TAA+EDTA	TAA+SeNa+EDTA
AST	0,0%	72,7%	85,4%	90,7%
ALT	0,0%	75,0%	87,3%	94,0%

In this study the SeNa therapy showed a small decrease in multifocal necrosis (absent 60%) and scarce passive chronic hyperemia. In contrast TAA group(I) shown multifocal necrosis present in 100% and passive chronic hyperemia increase suggesting that the exogenous administration of SeNa decrease but doesn't stop the damage caused by TAA.(7)

Low Selenium levels has been found in hepatic pathologies, therefore the low Selenium availability in volcanic soils as in Latin-American countries, might diminish this antioxidant advantages to local population´s diet.(14)(15)

In the group with EDTA necrosis was absent in 60% and the passive chronic hyperemia similar to SeNa group.

The synergic activity of EDTA and SeNa is shown in group IV, with 25% without hepatic damage and 75% with initial damage only. This was the only group with normal enzymatic values obtaining a 65.3% of hepatic protection. Combined therapy could contribute to diminish the FOR and FNR, increasing bioavailability of selenium and the activity of glutathione peroxidase thus diminishing hepatic damage.

These results provide evidence of using chelating agents and antioxidants in a therapy for hepatic failure. In a model using TAA as hepatic damage inductor, is not possible to completely avoid liver damage, however it is possible to limit the progression. Data from histopathology analysis and enzyme function (AST, ALT) suggest that combined therapy using EDTA and SeNa could be more effective in reducing the progression of hepatic damage. Given the antioxidant effect of SeNa by diminishing OS, increasing collagen degradation and reducing its production by reducing HSC activation.(10) This represents the most probable explanation to the limited hepatic injury with SeNa, improving this effect with EDTA supplementation as a chelating agent, diminishing reactive oxygen species and increasing antioxidant effects.

Histopathologic and enzymatic data reveals that the combination of EDTA and SeNa produce a better hepatoprotection response (65.3%) in contrast with monotherapy groups in the reduction of the progression in hepatic damage. This therapy represents an economic and vial option as supplementary treatment. And it represents better cost-benefit than other iron chelating therapies.

CONCLUSIONS

RECOMMENDATIONS FOR FURTHER INVESTIGATION

Employ EDTA and SeNa in other animal models of chronic hepatic injury.

Measure SeNa levels, serum proteins and indirect markers of hepatic disfunction like bilirubin and intrinsic coagulation pathways; as well as direct markers of OS like malondialdehyde.

Increase experimental groups sample.

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