Original Research

Comparison of Simvastatin and Nicotinic Acid Administration in Alcohol-Treated Wistar Rats

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Introduction: Previous studies of ours have shown that simvastatin (S) and nicotinic acid (NA) lower the alcohol (Alc)-induced increase of triglycerides. The aim of this study was to evaluate which drug is more effective and safe in decreasing Alc-induced hypertriglyceridaemia in Wistar rats.

Methods: Male Wistar rats were randomised into 6 groups, which were fed with: 1) olive oil (Oil group, n=10); 2) Oil + Alc, (Alc group, n=10); 3) S solution in Oil (65 μg/100g body weight), (S group, n=10); 4) NA solution in Oil (8.5 mg/100g body weight), (NA group, n=8); 5) S solution in Oil + Alc (S+Alc group, n=10); and 6) NA solution in Oil + Alc (NA+Alc group, n=9). Another 13 male Wistar rats were fed only a standard laboratory diet (control group). After 8 weeks, blood samples were drawn and the livers were removed. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), total cholesterol (TC) and triglycerides (TG) were measured. Liver histopathology was also assessed.

Results: Liver histopathology was similar in all groups and within the normal range. The TG plasma concentration in the Alc group was higher than in the control rats ($p<0.001$) or any other groups (Oil, $p<0.001$, or S, $p<0.001$, or NA, $p=0.003$). The Oil, S+Alc, NA+Alc and control groups had similar TG levels, but these were significantly lower compared to the Alc group ($p<0.001$). AST plasma concentration was higher in the Alc group compared to controls ($p<0.001$), Oil ($p<0.001$), S ($p<0.001$) and NA ($p<0.001$) groups, while the AST concentration in the S+Alc and Na+Alc groups was lower than in the Alc group ($p=0.042$, $p<0.001$, respectively).

Conclusions: NA and S, two drugs of different classes, seem to decrease Alc-induced secondary hypertriglyceridaemia to the same extent. Moreover, NA displays a better alleviation of Alc-induced AST raises compared to S, although it enhances small increases in AP and ALT levels.

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Large clinical trials have proved that hypolipidaemic treatment is able to reduce deaths from cardiovascular disease.[1,2] On the other hand, light to moderate alcohol (ethanol, Alc) consumption has been associated in many studies with a reduced risk of vascular events.[3-5] Alc itself may exert anti-inflammatory effects, for example on plasma C-reactive protein levels.[6] However, excessive Alc use can cause hyperlipidaemia,[7] fatty liver[8] and alcoholic liver cirrhosis, cardiomyopathy, hypertension, haemorrhagic stroke, cardiac arrhythmias or even sudden death.[9] Chronic Alc consumption may also lead to reduced drug metabolism.[10]

Simvastatin (S), an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and nicotinic acid (NA) are well-established agents in the treatment

of dyslipidaemia.[11-15] S lowers serum cholesterol levels by inhibiting hepatic cholesterol biosynthesis and thus upregulates hepatic low density lipoprotein (LDL) receptors, resulting in an increased uptake of LDL cholesterol from the blood and the subsequent lowering of circulating cholesterol levels.[16] Statins have anti-inflammatory and antiproliferative effects,[12] while they are the treatment of choice in patients with combined hyperlipidaemia. In some cases, statins produce characteristic alterations in liver histopathology, such as periportal hepatocellular atypia.[17] They have also been associated with hepatocellular necrosis in rabbits.[18]

NA, on the other hand, lowers plasma cholesterol and triglyceride (TG) levels by reducing very low density lipoprotein (VLDL) and LDL cholesterol levels.[14,15] Additionally, NA raises high density lipoprotein (HDL) cholesterol levels.[14,15] The administration of NA can be accompanied by adverse effects, which include flushing, itching, nausea, diarrhoea, decreased glucose tolerance, hyperuricaemia and hyperhomocysteinaemia.[19-21] Elevated hepatic enzymes, cholestasis and hepatocellular injury have also been observed in cases of NA administration.[22] The evidence suggests that NA-induced toxicity is dose-related.[23]

In previous studies of ours[24-26] S and NA were found to lower the Alc-induced increase of TG levels. A critical question that arises is which hypolipidaemic drug, S or NA, performs better in the case of concurrent Alc consumption. Therefore, we compared the administration of S with that of NA in Alc-treated Wistar rats.

Methods

Animals

All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals.[27] The use of animals was reviewed and approved by the animal care review committee of the Department of Experimental Pharmacology, Athens University. The Ethics Committee of that Department approved our study protocol. Seventy-three male normolipidaemic Wistar rats (8 weeks old at the start of the experiment) were purchased from the Hellenic Pasteur Institute, Athens, Greece. Our study protocol has already been described in our previous studies.[24-26] The rats were randomly assigned to six groups of 10 each and to a control group of 13 rats. The rats that served as controls were only fed a standard laboratory diet, while all the other rats were also stomach tube fed. All rat groups were fed for 2 months, as follows:

- Oil: tube fed with 2 ml of virgin olive oil (Oil). Virgin olive oil was chosen as a carrier for the lipophilic S and NA.
- Alc: tube fed with 2 ml of Oil and 2 ml of 25% v/v pure Alc in water.
- S: tube fed with 2 ml of S solution in Oil. Simvastatin tablets (Zocor 10 mg) were powdered and dissolved in Oil in order to achieve a final concentration of 65 μg/100 g body weight. The Zocor tablets were produced by Merck & Co., Inc. (Whitehouse Station, New Jersey, USA).
- Group NA: tube fed with 2 ml of NA solution in Oil. Nicolar tablets (Niacin 500 mg) were powdered and dissolved in Oil in order to achieve a final concentration of 8.5 mg/100 g body weight. The Nicolar tablets were produced by Aventis Pharmaceuticals Products, Inc. (Bridgewater NJ, USA).
- Group S+Alc: tube fed with 2 ml of S solution in Oil and 2 ml of 25% v/v pure Alc in water.
- Group NA+Alc: tube fed with 2 ml of NA solution in Oil and 2 ml of 25% v/v pure Alc in water.
- Control group: These animals were only fed the standard rat laboratory diet.

Dose preparation

The oral dose formulation of S was prepared in a 2 ml Oil solution, which included dissolved 0.13 mg of S, in order to achieve a minimal volume of administered liquid. The oral dose formulation of NA was prepared in a 2 ml Oil solution, which included dissolved 17 mg of NA, in order to achieve a minimal volume of administered liquid.

Blood samples

Blood for lipid and liver function tests was collected at the end of the study from all animals. Blood total cholesterol (TC), TG, alkaline phosphatase (AP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured.[28]

Liver histopathology was examined after fixing in buffered formalin and embedding in paraffin wax, using conventional techniques.[29,30] The possible Alc-, S- or NA-induced morphological changes in the rat liver were assessed. All animals were randomised and code numbers were given. Therefore, the examiner did not know the origin of the histology specimens or the blood samples.

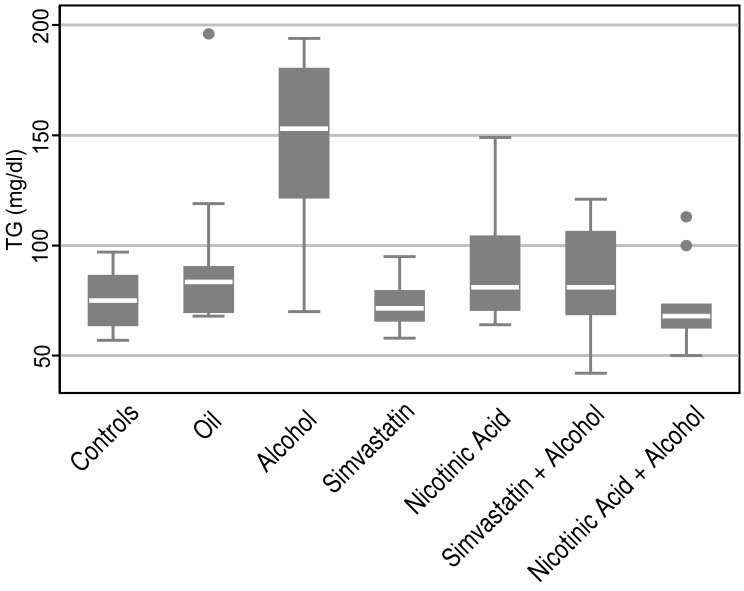

Figure 1. Triglyceride (TG) concentrations in all groups.

Statistical Analysis

Power analysis showed that the number of 10 rats in each study group was sufficient in order to evaluate two-sided differences greater than 15% in the investigated parameters, achieving a statistical power of 80% at the $p<0.05$ probability level. Values of numerical characteristics were tested for normality using the Shapiro-Wilk test. All variables deviated from normality; therefore, non-parametric statistical methods were used. The Mann-Whitney U test was used for the comparison of numerical values between two groups, while the Kruskal Wallis H test was used for the comparison of numerical values between the seven groups of the study. Continuous variables are presented as medians and interquartile ranges (IQRs, 75th - 25th percentile). All tests were two-sided and considered significant if $p<0.05$. Data were analysed using STATA™ (Version 9.0, Stata Corporation, College Station, TX 77845, USA).

Results

Two rats of the NA group and one rat of the NA+Alc group died at the beginning of the experiment for unknown reasons. This fact cannot be attributed to drug side effects, since their administration had just started. Moreover, there were no pathological findings during the autopsy.

After 2 months of treatment, the TC levels displayed no significant reduction in the drug-fed groups, but the Oil group had lower TC levels compared to controls ($p=0.004$), Alc ($p=0.007$) and S+Alc ($p=0.003$) groups (Table 1). The TG plasma concentration in the Alc group was significantly higher than in the control rats or in any other treatment group (Figure 1). The comparison between groups concerning AST plasma concentration is shown in Figure 2. The S+Alc group had higher AST levels than all the other groups except for Alc (controls, $p<0.001$, Oil, $p=0.002$, S, $p=0.021$, NA, $p=0.003$, and NA+Alc,

Table 1. Total cholesterol (TC), alkaline phosphatase (AP), and alanine aminotransferase (ALT) in all groups. Variables are presented as medians (IQRs).

Groups	TC (mg/dl)	AP (mIU/ml)	ALT (mIU/ml)
Controls (n=13)	60 (19.5)	60 (21)	60 (13)
Oil (n=10)	54 (6)	49.5 (16.5)	49 (9)
Alc (n=10)	57.5 (5.25)	53 (15)	53 (4.5)
S (n=10)	57.5 (22.25)	55 (11)	49.5 (17)
NA (n=8)	54 (14)	82 (13)	64 (10)
S+Alc (n=10)	62 (18.5)	51 (18)	52.5 (18.75)
NA+Alc (n=9)	53 (24.5)	77 (27)	59 (14)
Overall p-value	0.218	0.010	0.001

Alc – alcohol; NA – nicotinic acid; S – simvastatin.

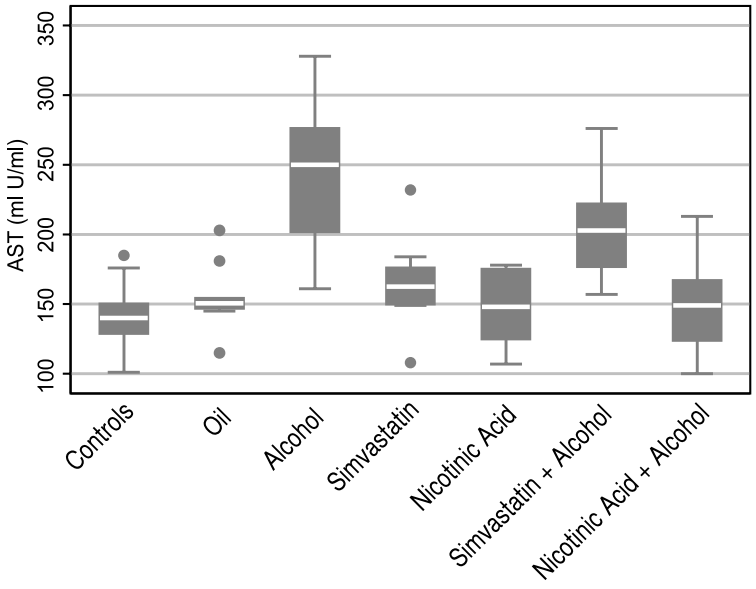

Figure 2. Aspartate aminotransferase (AST) concentrations in all groups.

p=0.006, respectively). The AP levels were significantly higher in the NA group compared to all other groups except for the controls (NA vs. Oil p=0.002, NA vs. Alc p=0.005, NA vs. S p=0.006, NA vs. S+Alc p=0.005, NA+Alc vs. Oil p=0.001, NA+Alc vs. Alc p=0.001, NA+Alc vs. S p=0.002, NA+Alc vs. S+Alc p=0.003) (Table 1). Additionally, the Oil, S and S+Alc groups had lower ALT concentrations compared to the controls (p=0.001, p=0.002, p=0.005, respectively) and to the NA+Alc group (p=0.001, p=0.002, p=0.007), while the NA group had a higher ALT concentration compared to Oil (p=0.002), Alc (p=0.005), S (p=0.004) and S+Alc (p=0.005) groups (Table 1). A significant difference was observed in the median weight between the rats given Alc+Oil and the control rats (298(8) vs. 313(9) g, p<0.001).

Liver histopathology was similar in all groups and within the normal range. Examination of the liver specimens disclosed normal hepatic structure and cytology. However, there were moderate variations within the normal histology.

Discussion

This study demonstrated that NA and S alleviate Alc-induced increase of TGs to the same extent. They also eliminated an Alc-induced AST increase, although NA displayed greater effectiveness. On the other hand, NA enhanced small increases in ALT and AP levels in contrast to S.

Alc is one of the factors most frequently associated with increased liver enzyme concentration.[24-26,31-32] Moreover, the association between Alc intake and Alc-induced liver disease is well known and was extensively discussed in our previous review.[33] In our study Alc administration caused an increase in AST levels, similar to that reported by Kamimura et al,[34] who observed two- and threefold increases in plasma ALT and AST levels in Alc-fed male Wistar rats. Other investigators have also demonstrated an elevation of aminotransferase levels in humans[31] and even a substantial one in alcoholic liver disease.[32] In opposition to our findings are those of Duk-Hee Lee et al,[35] who reported that serum levels of liver enzymes are affected by body mass index alterations, rather than alcohol consumption. According to those researchers, weight gain predominantly causes the rise in AST levels. In contrast, in our study, the Alc-fed rats gained less weight than the control rats but their AST levels were still elevated. The close relation between ethanol consumption and liver function is due to the fact that more than 80% of ingested Alc is metabolised in the liver without a feedback mechanism.[36] In the early phase, oxygen and NO-radicals derive from the complete oxidation of ethanol, and acetaldehyde in excess markedly alters the intracellular redox status, induces fat deposits, and triggers the inflammatory and immune response.[37] The progression of liver damage is also affected by the generation of additional products between acetaldehyde and cytochrome c oxidase and/or P450 2E1.[38,39]

Aminotransferases catalyse the transfer of an a-amino group from an a-amino acid to an a-keto acid. Serum AST concentration is associated[40] with mortality caused by liver disease, even within the current normal range. If Alc consumption is a common cause

of transaminase elevation,[31-32,34] its beneficial effect on cardiovascular events may be diminished. The dose of Alc in our study was moderate and the increase it caused in AST was eventually decreased by S, but particularly by NA action.

As stated above, NA was found to reduce the AST elevations induced by Alc more effectively than did S. On the other hand, NA caused a small elevation of ALT and AP levels. The increase of ALT levels by NA, though in a sustained-release formulation, has also been noticed by other investigators. Moreover, the NA-induced AP raises have also been found in studies of humans, where they were not considered biologically meaningful.[41]

Besides the elevation of liver enzymes, Alc also induces secondary hypertriglyceridaemia. The possible mechanisms of Alc-induced TG level increase have been discussed previously.[24-26,39] Such Alc-induced secondary hypertriglyceridaemia[42] was found to be alleviated to the same extent by either S or NA administration.

NA is known to inhibit adipose tissue lipolysis as well as hepatic TG, cholesterol and apolipoprotein B synthesis.[43] S is a statin which, in addition to its effect in decreasing plasma cholesterol, also has a hypotriglyceridaemic effect.[44] In the present study, no reduction of serum TG levels was observed when either NA or S were administered alone, and this can be partially attributed to the very low baseline TG levels. However, a significant reduction of TG levels that were apparently raised by Alc occurred in the case of NA or S coadministration with Alc. It seems that animals treated with Alc developed secondary dyslipidaemia which responded to hypolipidaemic treatment with NA or S, since both drugs affect TG plasma concentration. Despite the strict isocaloric pair-feeding, Alc-fed animals did not gain as much weight as their pair-fed controls, although they received diets with the same energy content.[45] This may be due to the oxidation of Alc without phosphorylation by the microsomal ethanol-oxidising system (MEOS). When Alc is oxidised to acetaldehyde via the alcohol dehydrogenase (ADH) pathway, NADH is generated. However, the oxidation of Alc via MEOS utilises NADPH, resulting in energy wastage as heat, which may explain the slower weight gain of the rats fed Alc-containing liquid diets. This delay in weight gain happened despite the similar calorific intake of all rats (fed with Alc or not) and the increase in weight that was expected to follow Alc withdrawal.[46] The slower weight gain of the animals in this study compared with that reported by other authors[47] could be attributed to strain and/or sex differences in the rats used, or to the conditions of housing, such as the room temperature.

Chronic excessive consumption of ethanol can cause hepatosteatosis, liver fibrosis and cirrhosis.[17] Also, statins can cause damage to the hepatic cells, mainly by periportal hepatocellular atypia (the periportal hepatocytes are hypertrophied and are eosinophilic) and hepatocellular necrosis (with higher doses). Thus, hepatotoxicity as a result of the coadministration of both could be more pronounced.[18] Nevertheless, in our study significant changes in liver histopathology were not found. This can be explained by the fact that, firstly, the dose of S was smaller than in other studies, and secondly, the amount of Alc intake by the animals was moderate. NA may also cause hepatotoxicity associated with raised hepatic enzymes, cholestasis and hepatocellular injury.[19-23] The evidence suggests that NA-induced toxicity is also dose related. The mechanism by which NA induces liver injury remains unclear. The hepatotoxic effects of sustained-release NA are characterised by centrilobular cholestasis and parenchymal necrosis.[22,23] Sustained-release formulations of NA are more associated with hepatotoxicity than crystalline preparations[21,22] like the one we used. This is one of the possible explanations why there were no changes in liver histopathology in our study. Although Alc consumption, as well as S and NA administration, have been implicated in hepatotoxicity, the rats in all of our study groups displayed normal liver histology. This finding supports the safety of the combination of hypolipidaemic treatment and Alc consumption in rats.

In conclusion, both S and NA seem to be effective in alleviating secondary hypertriglyceridaemia, and presumably its complications,[48] despite the different mechanisms of action, which cannot be accidental. Nevertheless, NA displays a more effective result in decreasing Alc-induced AST increase, though its administration triggers small ALT and AP elevations in contrast to S. Given the inverse correlation between TG and HDL-C levels in humans,[49] it is suggested that more research in this field, including studies with humans, is needed in order to determine whether the consumption of Alc in combination with drugs having hypotriglyceridaemic properties should be recommended.

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