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DELETERIOUS EFFECTS OF LOVASTATIN IN RATS FED DIETS DEFICIENT OR SUPPLEMENTED WITH VITAMIN E

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ABSTRACT

We have previously reported in this journal that prolonged dietary administration of lovastatin to vitamin E-deficient rats resulted in a dose-dependent mortality due to massive hepatic necrosis, and that vitamin E supplementation totally prevented mortality and reduced the liver damage. Since the results suggested that the lethal hepatic effects of lovastatin were due to oxidative stress, we have now reexplored this situation in more detail. Female weaning Wistar rats were fed either a diet deficient or supplemented with vitamin E, and these two regimens were offered for six weeks alone (controls) or supplemented with 200 or 400 mg of lovastatin/kg of diet. The results showed that in vit. E-def. rats treated with lovastatin the mortality was 40% at the dosage of 200 mg/kg, and 50% at the dosage of 400 mg/kg, while contrary to our expectations, in the vit. E-suppl. rats the mortality was 10% at the lovastatin dosage of 200 mg/ kg, and still 50% at the dosage of 400 mg/kg. This time the lethal effects of lovastatin could not be ascribed to any histologic evidence of severe liver necrosis. At the dosage of 200 mg/kg lovastatin significantly reduced plasma contents of α-tocopherol, and significantly increased the serum levels of ALT and AST in the surviving vit. E-def. rats, but not in those supplemented with this vitamin. On the other hand, at the dosage of 400 mg/kg lovastatin decreased the plasma contents of α-tocopherol and β-carotene, but increased plasma ubiquinol-9 and did not affect serum ALT or AST in vit. E-def. rats. In vit. E-suppl. rats the only significant change associated with lovastatin at 400 mg/kg was a decrease in plasma α-tocopherol. In none of the surviving rats lovastatin treatment increased the liver spontaneous and hydroxyperoxyde-induced chemiluminescence or the production of thiobarbituric acid reactive substances. Although the present results in surviving rats do not apparently support our "oxidative stress" hypothesis of lovastatin toxicity, they strongly suggest that rats may have adapted to the untoward effects of this drug.

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(12) and expressed as counts per second/mg of homogenate protein. In vitro production of thiobarbituric acid reactive substances (13) and the contents of α-tocopherol (11) were also determined in the hepatic homogenates. Other portions of the livers were fixed in buffered formalin and frozen sections were stained with oil red O (14) for the detection of fat. Deparaffinized sections were stained with H & E, and with PAS (with and without previous diastase digestion) for the detection of the ceroid by conventional light microscopy. The amounts of ceroid pigment accumulated in hepatocytes and Kupffer cells were blindly evaluated semiquantitatively by two observers. Animals dying during the course of the experiment were autopsied and blocks of liver tissue were processed for histopathologic studies as above. Statistical analysis of the numerical data was done by the Duncan's multiple range test (15).

RESULTS AND DISCUSSION

Mortality (Table 1)

None of the untreated rats fed either the vitamin E-deficient or supplemented diets died during the course of the experiment. In the group of rats fed the vitamin E-deficient diet containing 200 mg/kg of lovastatin the mortality was 40%, while in the group receiving the vitamin E-supplemented diet with the same concentration of lovastatin the mortality was 10%. In rats fed the vitamin E-deficient or supplemented diets containing 400 mg/kg of lovastatin the mortality was 50%. Thus, although the dietary supplementation of vitamin E reduced the mortality observed in vitamin E-deficient rats treated with lovastatin at the concentration of 200 mg/kg diet, it did not modify the 50% mortality when the lovastatin concentration was 400 mg/kg of diet. These results differed somewhat from those of our previous study (4) in which the dietary supplementation with vitamin E totally prevented the 50% mortality observed in vitamin E-deficient rats treated with lovastatin at the concentration of 500 mg/kg of diet. Although we don't know the reason(s) for this difference, it is possible that in the present study the addition of sodium selenite, albeit at a non-toxic level, to the vitamin E-supplemented diet might have had a synergistic effect with lovastatin.

In all the groups of rats treated with lovastatin the mortality mainly occurred during the first three weeks of the experiment and, therefore, although the lethal effects of lovastatin were apparently dose-dependent, they were not time-dependent. This strongly suggests that the surviving animals have in some way adapted to lovastatin toxicity. Like in any toxicological study the surviving animals represent the fittest or best adapted ones and this usually helps to interpret the low or lack of effects of a given toxic dosage in the survivors. Further studies with sequential analysis of the parameters indicative of liver damage may show whether the surviving lovastatin-treated rats have really adapted to this drug.

Food Intakes (Table 1)

The data presented in Table 1 correspond only to rats surviving the whole experimental period. In untreated rats fed the diet deficient in vitamin E the average relative daily food intake was statistically similar to that of the rats fed

TABLE 1
BODY WEIGHTS, DAILY GROWTH, FOOD INTAKE AND MORTALITY DATA

Treatment	Body Weights Initial (g)	Final (g)	Daily Growth (g/day)	Food Intake (g/day/100 g b. wt.)	Mortality (%)
Vitamin E-Deficient Rats					
Untreat.	44.4 ± 1.0(10)[1]	89.9 ± 3.2(10)	1.08 ± 0.08(10)	11.10 ± 0.42(10)	0
Lovast. 200 mg/kg	44.9 ± 0.7(10)	56.7 ± 4.1(6)[2]	0.28 ± 0.10(6)[2]	12.73 ± 0.43(6)[2]	40
Lovast. 400 mg/kg	44.8 ± 0.8(10)	55.6 ± 4.1(5)[2]	0.28 ± 0.09(5)[2]	12.81 ± 0.45(5)[2]	50
Vitamin E-Supplemented Rats					
Untreat.	44.7 ± 0.8(10)	70.4 ± 2.3(10)	0.61 ± 0.06(10)	11.50 ± 0.31(10)	0
Lovast. 200 mg/kg	44.3 ± 0.9(10)	66.6 ± 2.3(9)	0.52 ± 0.16(9)	12.51 ± 0.37(9)[2]	10
Lovast. 400 mg/kg	44.6 ± 0.7(10)	74.6 ± 4.6(5)	0.73 ± 0.10(5)	12.98 ± 0.57(5)[2]	50

[1]Mean ± SEM. [2]Significantly different from the corresponding untreated control rats. Number of rats in parenthesis.

rats treated with lovastatin consumed, in fact, significantly higher amounts of food than the corresponding untreated control rats. Thus, the toxicity of lovastatin was not only reflected in the mortality rates but also in its decreasing effect on body weight.

Activity of Serum Aminotransferases (Table 2).

In untreated rats the dietary deficiency of vitamin E did not statistically affect the serum levels of ALT activity, but significantly increased by 52% the activity of AST. This increase may not necessarily indicate liver damage and it is most probably mainly due to the myopathy described in vitamin E-deficient rats

cing effect of lovastatin treatment on serum trans-β-carotene levels was comparatively much lower than on the serum levels of α-tocopherol. If the reductions of circulating vitamin E associated with lovastatin treatment was really due to a prooxidant effect of this drug (or its metabolites) on the plasma lipoproteins carrying vitamin E, as well as the carotene, the relative resistance of the β-carotene may be expected on the basis of previous studies. It has been shown in this respect that while vitamin E is destroyed or converted to quinones in the presence of prooxidants (27,28), the β-carotene remains stable and is recycled (29,30). Further studies are clearly needed to determine whether or not the lovastatin treatment may produce an oxidative stress in the blood.

In untreated rats fed the diet deficient in vitamin E the plasma levels of ubiquinol-9 were significantly reduced by almost 43 % in relation to the values in vitamin E-supplemented animals. A significant reduction of ubiquinols has been also found by others in the blood, liver and other tissues of vitamin E-deficient male and female rats (31-33). The reason for these reductions in ubiquinol in the plasma and other tissues of vitamin E-deficient rats is presently unknown.

In rats deficient in vitamin E the lovastatin treatment at the dietary concentration of 400 mg/kg significantly increased the circulating levels of ubiquinol-9. Although in rats fed the diet supplemented with vitamin E the lovastatin treatment at the dietary concentrations of 200 and 400 mg/kg reduced the plasma ubiquinol-9 by 4% and 19%, respectively, these reductions were not statistically significant in relation to the values of the corresponding non-treated control rats. It has been recently reported by Willis et al. (34) that the administration of lovastatin (400 mg/kg diet [Purina chow]) for 4 weeks to male Holtzman adult rats (480 g) significantly reduced the circulating and liver concentrations of ubiquinol-9 by 33% and 30%, respectively. Although our results are somewhat different, in the sense that we could not statistically confirm the significance of the lovastatin-reducing effect on circulating levels of ubiquinol-9, this relatively minor discrepancy should be addressed with respect to several procedural differences. First, we have used younger rats of different sex and strain (female Wistar, ~44 g). Second, although the dietary concentration of lovastatin was similar in both studies, the composition of the basal diet consumed by our rats was substantially different and contained higher amounts of vitamin E (160 mg/kg versus ~ 37 mg/kg). Third, the number of animals per group was much smaller than in the study of Willis et al. (34). Finally, while in the study of Willis et al. (34) the duration of lovastatin treatment was 4 weeks, in the present experiment it was six weeks. It is worth mentioning here that a significant decrease in the circulating levels of ubiquinol-10 was also reported by Folkers et al. (35) in patients treated with lovastatin (20-40 mg/day), and by Mabuchi et al. (36) in patients treated with compactin (similar to lovastatin but lacking the methyl group at position 6 of the decaline ring). Furthermore, lovastatin treatment also significantly reduced the concentration of ubiquinol in cardiac muscle and cardiac mitochondria of guinea pigs (37), as well as in brain homogenates of dogs (19). Lovastatin reversibly inhibits HMG-Co A reductase, the rate limiting enzyme catalyzing the conversion of HMG-Co A to mevalonate, which in turn is an early precursor in the biosynthesis of not only cholesterol, but also other isoprenoids, such as ubiquinone, dolichol and isopentenyl adenine (38).

Thus, all the available evidence indicates that lovastatin and other inhibitors of this reductase are capable to decrease the synthesis of ubiquinols in man and experimental animals.

Hepatic Concentrations of α-Tocopherol (Table 3)

As expected, the hepatic concentration of α-tocopherol in untreated rats was significantly lower (by 35%) in the vitamin E-deficient animals than in those supplemented with this vitamin. Although in the livers of rats deficient or supplemented with vitamin E and treated with lovastatin at the concentration of 200 or 400 mg/kg the levels of α-tocopherol were always lower than in their respective untreated control rats, these reductions were not statistically significant. It seems, therefore that the reducing effect of lovastatin treatment on the vitamin E content is comparatively more pronounced in the plasma than in the liver. It should be noted again that the hepatic concentrations of α-tocopherol, as well as all the other biochemical determinations, were performed in the surviving animals and not in those succumbing by the lovastatin treatment. Further sequential studies with larger number of rats are necessary to determine whether the hepatic levels of α-tocopherol are significantly reduced in the animals dying by the effect of lovastatin .

Hepatic Chemiluminescence (Table 3)

In untreated rats the dietary deficiency of α-tocopherol did not significantly affect the spontaneous or the tert-butyl hydroperoxide stimulated hepatic chemiluminescence in relation to that in rats fed the vitamin E-supplemented diet. Although it has been reported that in weanling Wistar rats fed a vitamin E-deficient diet for more than 2 weeks the spontaneous hepatic chemiluminescence was significantly higher than in the supplemented controls, the animals have been fed a Torula yeast-based diet containing cod liver oil and very low amounts of sulphur amino acids (39). It has been demonstrated in this regard that fish oil diets induce in rats significantly higher hepatic oxidative stress than corn oil diets (40). It is also noted that while the hepatic chemiluminescence in rats fed the Torula yeast-based diet supplemented with vitamin E was 43 ± 3 c.p.s./cm^2 (39), in our rats fed the casein-based diet containing corn oil and supplemented with this vitamin the chemiluminescence was only 24 ± 6 c.p.s./cm^2. It seems, therefore, that in order to elicit a significant increase in hepatic chemiluminescence the deficiency of vitamin E must be associated with dietary regimens with strong prooxidant properties.

Although lovastatin treatment at the concentration of 200 mg/kg of diet increased by 85% the spontaneous hepatic chemiluminescence in vitamin E-deficient rats and by 17% in vitamin E-supplemented rats over the values of the respective untreated control rats, these increases were not statistically significant. While lovastatin treatment at the concentration of 200 mg/kg also increased the tert-butyl hydroperoxide initiated hepatic chemiluminescence in

TABLE 3

HEPATIC α-TOCOPHEROL CONCENTRATION, CHEMILUMINESCENCE, PRODUCTION OF THIOBARBITURIC ACID REACTIVE SUBSTANCES AND MAIN HISTOLOGIC FINDINGS IN SURVIVING RATS

Treatm.	α-Tocopherol (nmol/g)[1]	Chemiluminescence[1] c.p.s./cm²	c.p.m./mg prot	TBARS μmol/g	C. Swelling[2]	Ceroid[2] Kupffer	Hepatocytes
Vitamin E-Deficient Rats							
Untreat.	5.23±0.14(7)[3]	20±4(9)	48,300±2,800(9)	0.14±0.01(10)	1.40±0.22(10)	1.72±0.25(10)	1.88±0.36(10)
Lovast. 200 mg/kg	4.45±0.35(2)	37±10(3)	59,000±6,500(4)	0.14±0.02(5)	1.40±0.24(6)	1.80±0.40(6)	2.10±0.33(6)
Lovast. 400 mg/kg	4.87±0.12(3)	20±4(4)	54,000±7,800(4)	0.14±0.01(5)	1.50±0.16(5)	1.50±0.27(5)	1.70±0.37(5)
Vitamin E-Supplemented Rats							
Untreat.	8.06±0.90(7)	24±6(7)	53,200±4,800(8)	0.10±0.01(10)	1.90±0.20(10)	1.27±0.23(10)	1.44±0.44(10)
Lovast. 200 mg/kg	7.24±0.27(8)	28±4(8)	51,600±3,100(9)	0.09±0.01(9)	1.33±0.18(9)	1.66±0.22(9)	1.72±0.12(9)
Lovast. 400 mg/kg	5.27±1.07(3)	19±4(3)	61,200±6,900(4)	0.11±0.02(5)	1.30±0.20(5)	1.40±0.18(5)	1.10±0.10(5)

[1]First column under chemiluminescence corresponds to the spontaneous emission from the intact liver, and second column corresponds to the tert-butyl hydroperoxide initiated emission from liver homogenates.
[2]Histopathologic grading is as follows: 1= minimal; 2= moderate; 3= severe.   [3]Mean ± SEM.

however, at dosage levels several times higher (25 mg/kg body weight/day) than the therapeutic range of human doses (0.4 to 1.6 mg/kg/day) (2).

Summary Statements and Concluding Remarks

The results of these studies indicated that the consumption of the vitamin E-deficient diet per se (in rats not treated with lovastatin) significantly decreased the plasma levels of α-tocopherol and ubiquinol-9, as well as the hepatic levels of α-tocopherol, and increased the hepatic lipid peroxidative potentials (increased TBARS). Although some of these data suggested that the liver of the deficient rats might have been affected by oxidative stress, no hepatic injury could be conclusively demonstrated (i.e. while the activity of serum AST was significantly elevated, that of ALT was not, and the evaluation of histopathologic hepatic changes showed no significant differences with those observed in the control rats supplemented with vitamin E). The deficient regimen per se did not produce any mortality.

In vitamin E-deficient rats the lovastatin treatment at the dietary concentration of 200 mg/kg resulted in 40% mortality. In the surviving rats the lovastatin treatment (200 mg/kg) was associated with significant decreases in body weight gains and plasma levels of α-tocopherol, and with significant increases in the serum activity of both alanine and aspartate aminotransferases suggestive of liver damage. In these deficient animals the lovastatin treatment at the dietary concentration of 400 mg/kg produced a 50% mortality, while the surviving rats showed significant decreases in body weight gains and in the plasma levels of α-tocopherol and β-carotene. However, the serum activities of both transaminases were not elevated and a significant increase was observed in the plasma levels of ubiquinol-9. These seemingly paradoxical results observed in the surviving animals may be perhaps explained by adaptive mechanisms against lovastatin toxicity. First, it is noted that the mortality associated with lovastatin treatment occurred almost exclusively during the first 3 weeks. Second, although at the concentration of 400 mg/kg the lovastatin treatment was associated not only with a decrease in the plasma levels of α-tocopherol but also in those of β-carotene, it was on the other hand associated with an increase in the plasma levels of ubiquinol-9, a well recognized antioxidant. Third, conversely to what it was observed in the vitamin E-deficient rats treated with lovastatin at the concentration of 200 mg/kg, in those treated with a much higher concentration of this drug (400 mg/kg), the activities of serum transaminases were not elevated. Finally, while the vitamin E-deficient diet per se produced some evidence of hepatic oxidative stress, this evidence was not present in the vitamin E-deficient rats treated with lovastatin at the concentration of 400 mg/kg. Since it has been repeatedly demonstrated that rats do adapt to oxidative stress (45-48), we have further reasons to suspect that most of the disconcerting biochemical findings in the present study may be due to adaptive mechanisms.

In vitamin E-supplemented rats the lovastatin treatment at the dietary concentration of 200 mg/kg resulted in 10% mortality and in a significantly decreased body weight gained in the survivors, but this obvious toxicity was not

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