Biochemical Pharmacology, Vol. 41, No. 3, pp. 460–461, 1991.
Printed in Great Britain.

0006-2952/91 $3.00 + 0.00

Serum pseudocholinesterase activity in rabbits fed simvastatin

(*Received* 25 *June* 1990; *accepted* 15 *September* 1990)

There is a variety of esterases in the serum and tissues of vertebrate animals [1]. One of the esterases in serum catalyses the hydrolysis of acetylcholine, but has properties different from acetylcholinesterase in tissues. This serum enzyme is indicated as pseudocholinesterase (EC 3.1.1.8), but is also known as cholinesterase or butyrylcholinesterase. The biological function of pseudocholinesterase has not been clearly established, but there is evidence that this enzyme is involved in serum cholesterol metabolism [2]. In the course of a study on modulation of cholesterol synthesis and serum cholesterol concentrations in rabbits, we measured pseudocholinesterase activity in serum. The data suggest that simvastatin (formerly synvinolin), a competitive inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase and potent serum cholesterol-lowering drug, lowers pseudocholinesterase activity in rabbits.

Materials and Methods

Male specified-pathogen free New Zealand White rabbits (Iffa Credo/Broekman Institute, Helmond, The Netherlands) were used. The rabbits, aged about 10 weeks, were housed individually as described previously [3]. For 3 weeks, all animals were fed a cholesterol-free, purified control diet. Then, on day 0 of the experimental period, the rabbits were divided into one control and three experimental groups, consisting of eight rabbits each. The four groups had similar distributions of body weight and serum cholesterol; the mean values were 2.75 kg and 2.6 mM. The control animals remained on the cholesterol-free diet and the other animals were transferred to diets containing either 0.1% (w/w) cholesterol, 0.03% (w/w) simvastatin (Merck, Sharpe & Dohme Inc., Rahway, NJ, U.S.A.) or 1% (w/w) cholestyramine (Mead Johnson & Co., Evansville, IN, U.S.A.). The latter two compounds are widely used to lower serum cholesterol concentrations.

The composition of the cholesterol-free control diet was (g/kg): soybean protein isolate, 158; methionine, 2; corn starch : dextrose (1 : 1, w/w), 418.3; cellulose, 150; molasses, 100; corn oil, 10; coconut fat, 90; calcium carbonate, 10; sodium dihydrogenphosphate, 5; sodium chloride, 5; sodium carbonate, 10.3; magnesium carbonate, 1.4; potassium bicarbonate 18; mineral premix, 10; and vitamin premix, 12. The compositions of the vitamin and mineral premixes have been described previously [3]. Cholesterol, simvastatin and cholestyramine were added to the diets at the expense of cellulose. The diets were in pelleted form. The rabbits were fed 75 g of food each day; tap water was provided *ad lib*. The experiment lasted 6 weeks.

Samples of blood were taken from the marginal ear vein of the rabbits. Blood was allowed to clot for 30 min at room temperature. Serum was collected by low-speed centrifugation and kept at −20° until analysis. Serum cholesterol [3] and pseudocholinesterase activity [4] were determined as described.

Results are presented as means ± SE. The Kolmogorov–Smirnov one-sample test was used to check normality of the data. Student's one-sample *t*-test for paired data was used to evaluate within-group changes with time. Differences between groups were evaluated by two-tailed Student's *t*-test (for equal variances) or two-tailed Welch's *t*-test (for unequal variances). The equality of variances was tested using a two-tailed F-test. In order to take into account the increased probability of a type I error due to multiple comparisons, the level of significance was pre-set at $P < 0.017$ instead of $P < 0.05$ (Bonferroni adaptation).

Results and Discussion

The experimental diets did not significantly influence body weight gain (data not shown). At the end of the experimental period, dietary cholesterol had increased liver and serum cholesterol on average by 153 and 258%, whereas these concentrations were decreased by 48 and 47%, and 45 and 44%, respectively, by cholestyramine and simvastatin. These results have been published in abstract form [5]. Although not statistically significant, initial pseudocholinesterase activities in the control animals were higher than those in the experimental animals (Table 1). Therefore, relative changes of pseudocholinesterase activities during the experimental period were calculated. In the control rabbits there was a slight but significant drop of pseudocholinesterase activity. The change of esterase activity in the animals fed cholesterol or cholestyramine did not differ from that in the control rabbits. However, when compared with the controls, in the rabbits fed simvastatin pseudocholinesterase activity was reduced by about 10%, this effect being statistically significant.

It could be hypothesized that the observed depression of serum pseudocholinesterase activity is caused by a direct, non-competitive or uncompetitive, inhibitory effect of simvastatin. Therefore, we have carried out kinetic studies with pseudocholinesterase in pooled rabbit serum to which

Table 1. Plasma pseudocholinesterase activities in rabbits fed purified diets for 6 weeks containing either cholesterol, simvastatin or cholestyramine

	Plasma pseudocholinesterase activity (nmol/min/mL)			
	Control	Cholesterol	Simvastatin	Cholestyramine
Initial	671 ± 138	559 ± 166	548 ± 115	510 ± 157
Final	604 ± 123	592 ± 178	454 ± 96	488 ± 138
Change (%)	−7.4 ± 3.0*	+3.9 ± 9.3	−18.3 ± 2.6*†	−3.8 ± 6.0

Results, expressed as means ± SE for eight animals per group.
* Significantly different from zero (two-tailed paired Student's *t*-test; $P < 0.05$).
† Significantly different from group fed the control diet (two-tailed Student's *t*-test; $P < 0.017$).

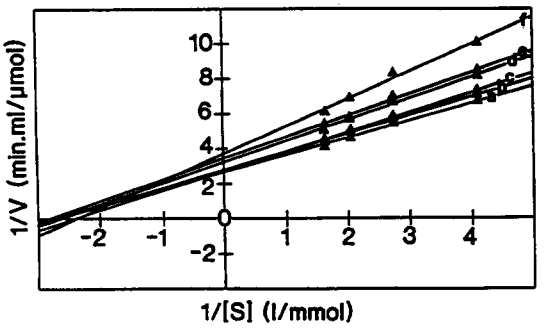

Fig. 1. Effect of simvastatin on pseudocholinesterase in rabbit serum. All points are the mean of three measurements; the straight lines were fitted by regression analysis. Simvastatin (µM) and dimethylsulphoxide (mM) concentrations were: (a) 0.0 and 0.0; (b) 0.0 and 127.5; (c) 0.4 and 127.5; (d) 2.2 and 127.5; (e) 10.9 and 127.5; (f) 21.9 and 127.5.

four different concentrations of simvastatin were added. Simvastatin was dissolved in dimethylsulphoxide before adding to the assay mixture. This amount of dimethylsulphoxide added (127.5 mM) did not influence pseudocholinesterase activity. The results were plotted as Lineweaver–Burk plots (Fig. 1). The linearity of these plots indicates Michaelis–Menten type kinetics. Pseudocholinesterase was inhibited by concentrations of simvastatin as low as 2.2 µM. At 21.9 µM simvastatin pseudocholinesterase was markedly inhibited. This assay concentration corresponds to the maximum concentration that can be attained in the serum of rabbits fed 0.03% (w/w) simvastatin. Simvastatin intake was calculated to be 55.6 µmol per rabbit and total serum volume of a rabbit was assumed to be 121 mL, the serum being diluted by a factor 21 in the final assay mixture.

The enzyme kinetic data were analysed according to Eisenthal and Cornish-Bowden [6], to avoid problems of error distortion arising from unweighted regression lines in the double-reciprocal plot [7]. The median values of the apparent K_m and V_{max} thus obtained were used to construct secondary replots (plots not shown) of K_m/V_{max} (corresponding to the slope of the Lineweaver–Burk plot) or $1/V_{max}$ (corresponding to the y-intercept of the Lineweaver–Burk plot) against simvastatin concentration. Both the slope and the y-intercept of the double-reciprocal plot were increased by simvastatin, indicating combined competitive and non-competitive inhibition of serum pseudocholinesterase by simvastatin. The calculated percentages of inhibition of pseudocholinesterase by simvastatin were: 0.4 µM, 2.1%; 2.2 µM, 12.6%; 10.9 µM, 23.5%; and 21.9 µM, 23.5%.

The observed *in vivo* decrease of pseudocholinesterase activity in rabbits fed simvastatin may be caused by non-competitive inhibition of the enzyme by the hypocholesterolemic drug as demonstrated *in vitro*. The *in vivo* decrease was of the same order of magnitude as the decrease seen *in vitro* with realistic simvastatin concentrations. It cannot be excluded that simvastatin ingestion lowers the number of pseudocholinesterase molecules in serum. In any event, our results do not support the idea [2] that pseudocholinesterase is involved in serum cholesterol metabolism. In rabbits fed either cholesterol, simvastatin or cholestyramine there was no association between the changes in serum cholesterol concentrations and pseudocholinesterase activities. In contrast to the present study with rabbits, simvastatin did not influence the activity of pseudocholinesterase in serum of patients with familial heterozygous hypercholesterolemia [8]. Possibly, this relates to the different doses given to the rabbits and the patients. The rabbits received about 10 mg of simvastatin/kg metabolic body weight/day, whereas for the patients this amount was about five times as low.

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