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Synergistic Effect of Squalene and Simvastatin on Fecal Cholesterol Excretion in Rats

K.H.S. Farvin, A. Surendraraj and R. Anandan
Biochemistry and Nutrition Division, Central Institute of Fisheries Technology,
Matsyapuri, Cochin-682029, India

Abstract: In the present study an attempt was made to study the synergetic effect of squalene and simvastatin on fecal cholesterol excretion. The rats were fed with 2% squalene and/or 20 mg simvastatin for 45 days. At the end of the experimental period the feces were collected and analyzed for total lipid and total cholesterol content. It was found that the dietary supplementation of squalene at 2% level significantly ($p < 0.001$) increased the fecal cholesterol excretion in experimental rats. The combination of squalene and simvastatin was found to be more efficient than squalene alone. Thus the results of the present study indicate that a combination of squalene and simvastatin can be efficient in cholesterol lowering than the drugs alone. It suggests that squalene a prime HMG CoA reductase inhibitor can also be used as a prudent dietary addition for individuals using similar cholesterol lowering drugs.

Key words: Squalene, simvastatin, fecal cholesterol excretion

INTRODUCTION

Squalene, a key intermediate in cholesterol biosynthesis, is present in shark liver oil in higher quantities (Thankappan, 2003). It also present at lower concentration in olive oil (0.2-0.7%) and a variety of other food stuffs (Ryan *et al.*, 2007). It is the principal hydrocarbon of human surface lipids amounting up to 11% of total surface fat, where it protects the skin from harmful Ultra Violet (UV) radiation (Ohsawa *et al.*, 1984). It is used as an effective chemopreventive agent against a variety of cancers, skin disorders and liver diseases (Gregory and Kelly, 1999). Recently, the preventive effect of squalene on myocardial infarction is established by Farvin *et al.* (2004).

Squalene stimulates acyl-coenzyme A cholesterol acyltransferase (ACAT, EC 2.3.1.26) activity but at the same time it is a feed back inhibitor of the enzyme hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) which has been the target of a class of cholesterol-lowering drugs (Strandberg *et al.*, 1989). Although, oral administration of squalene appears to decrease hepatic and serum levels of sterols consistently, its impact on cholesterol metabolism is less clear. Qureshi *et al.* (1996) have reported that squalene administration to one-day-old chicks lowers the serum cholesterol levels. Reports of Miettinen and Vanhelen (1994) indicated that dietary supplementation of squalene for

Corresponding Author: K.H. Sabeena Farvin, Section for Aquatic Lipids and Oxidation,
National Institute of Aquatic resources (DTU-AQUA),
Technical University of Denmark, B. 221, Søltøfts Plads, DTU,
DK-2800 Kgs., Lyngby, Denmark Tel: + 45 45 25 25 42
Fax: +45 45 88 47 74

9 weeks increases the serum total, VLDL, IDL and LDL cholesterol concentrations. In human, supplementation with squalene (900 mg day⁻¹ for 7-30 days) is found to raise serum squalene levels (17-fold) without any significant changes in triglycerides and cholesterol contents (Strandberg *et al.*, 1990). The compensatory mechanisms to prevent the increase of serum cholesterol during moderate long-term squalene consumption are reduced HMG-CoA reductase activity and increased fecal elimination of cholesterol (Standberg *et al.*, 1990). Earlier an attempt has been made by Chan *et al.* (1996) to compare pravastatin (10 mg), a hyperlipidemic drug and squalene (860 mg) either alone or in combination for the treatment of hypercholesterolemia. They found that pravastatin is more effective than squalene in reducing total cholesterol, LDL cholesterol and triglyceride and in increasing levels of HDL cholesterol in plasma. But the combination of both pravastatin and squalene is more effective than pravastatin or squalene alone. However, there is no report on the effect of statins, on the fecal cholesterol excretion. So, a study has been undertaken to evaluate the comparative effect of squalene and simvastatin, a known cholesterol-lowering drug in the fecal excretion of cholesterol.

MATERIALS AND METHODS

Drugs and Chemicals

Simvastatin was obtained from Ranbaxy laboratories India Ltd., Gurgaon, India. Squalene (Specific gravity: 0.853; Refractive index: 1.493; Saponification value: 30; Iodine value: 344; Boiling point: 240-245°C) was prepared from the shark liver oil of *Centrophorus* sp., caught in the Andaman waters (Farvin *et al.*, 2004). All the other chemicals used were of analytical grade.

Animals

Male Wistar strain albino rats, weighing 120-150 g were selected for the study. The animals were housed individually in polypropylene cages under hygienic and standard environmental conditions (28±2°C, humidity 60-70%, 12 h light/dark cycle). The animals were fed with a standard diet (M/s Sai Feeds, Bangalore, India) and water *ad libitum*. The experiment was carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethics Committee (IAEC).

Experimental Protocol

Seven days after acclimatization, the animals were divided into four groups of 6 rats each. Group I animals were fed on standard diet with added coconut oil at 2% level for 45 days, group II animals were fed on standard diet with added squalene at 2% level for 45 days, group III animals were fed on standard diet with added simvastatin at 20 mg for 45 days and group IV animals were fed on standard diet with added 2% squalene and 20 mg simvastatin for the same period. Feces were collected in last 10 days of the experimental period. The feces were dried and powdered.

Biochemical Analysis

The total lipid was extracted from the feces samples by using the method of Folch *et al.* (1957). In brief: 5 g of the powdered feces sample was subjected to lipid extraction twice by using 25 volume of chloroform-methanol mixture (2:1). The lipid extracts were transferred to a separating funnel and added 0.2 volume of water and left overnight. Next day

the lipid extracts along with the chloroform were drained through filter paper containing anhydrous sodium sulphate and was collected in round bottom flask. The chloroform was evaporated off by using a flash evaporator. The lipid in the round bottom flask was made up to 10 mL by using chloroform. From this 1.0 mL was taken into a pre-weighed vial and allowed to dry in warm temperature to constant weight and total lipid content were calculated from the difference in weight.

Cholesterol content was estimated by the method of Parekh and Jung (1970). In brief: 0.1 mL of the lipid sample was taken into a 25 mL glass stoppered tube and evaporated off the chloroform. Mixed well with 5 mL of freshly prepared alcoholic KOH solution and incubated in a water bath at 37°C for 55 min. After cooling to room temperature, 10 mL of petroleum ether was added and inverted the tubes to mix the contents. To this 5.0 mL of distilled water was added and the tubes were shaken vigorously for 1 min. An aliquot (0.5-2 mL) of the supernatant was taken into test tubes and petroleum ether was evaporated under nitrogen. To each of the samples 3.0 mL of glacial acetic acid and 0.1 mL distilled water was added and mixed thoroughly. To this added 2 mL of the FeCl₃-H₂SO₄ reagent (2.0 mL of 10% FeCl₃ in acetic acid was diluted to 200 mL with concentrated H₂SO₄) through the sides of the test tubes to form a brown ring at the interface. Bottom of the tubes were tapped well to effect mixing and a light colour appeared which changed to an immense purple colour, was measured in a Shimadzu-UV spectrophotometer at 560 nm. A blank without sample and a standard with different concentration of cholesterol was also run in the same manner to obtain a calibration curve. The cholesterol content was expressed as mg g⁻¹ of sample.

Statistical Analysis

Mean values of each parameter tested were calculated and the results were expressed as mean±SD of 6 animals. One way Analysis of Variance (ANOVA) was carried out and the statistical comparisons among the groups were performed with Tukey's test using a statistical package program (SPSS 10.0 for windows). A p-value<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The present study examined the combined effect of both dietary squalene and cholesterol lowering drug simvastatin on the fecal excretion of cholesterol. Figure 1a and b shows the total lipids and cholesterol content in the feces of the normal and experimental groups of rats. After 45 days of feeding, significant (p<0.001) elevation was observed in the fecal excretion of lipids and cholesterol in Group II squalene administered rats compared to Group I control rats. Squalene feeding has been reported to increase fecal excretion of cholesterol, its non-polar derivatives and bile acids (Tilvis and Miettinen, 1983). Present study also strongly suggests that a considerable amount of the administered squalene is absorbed from the intestine and a part of it was metabolized to bile acids via cholesterol and excreted into feces. There was no significant (p<0.001) change in the excretion of lipids and cholesterol in Group III simvastatin treated animals compared to group I control rats (Fig. 1a, b). This indicates that simvastatin, lower serum cholesterol in a mechanism other than cholesterol excretion. Squalene was much potent than simvastatin in enhancing the excretion of cholesterol. One of the reasons for the antilipidemic property of the squalene might be due to the increased elimination of the lipids and cholesterol through feces. Although, cholesterol synthesis increased, fecal elimination was also up regulated resulting in no significant difference on serum cholesterol concentrations (Strandberg *et al.*, 1989).

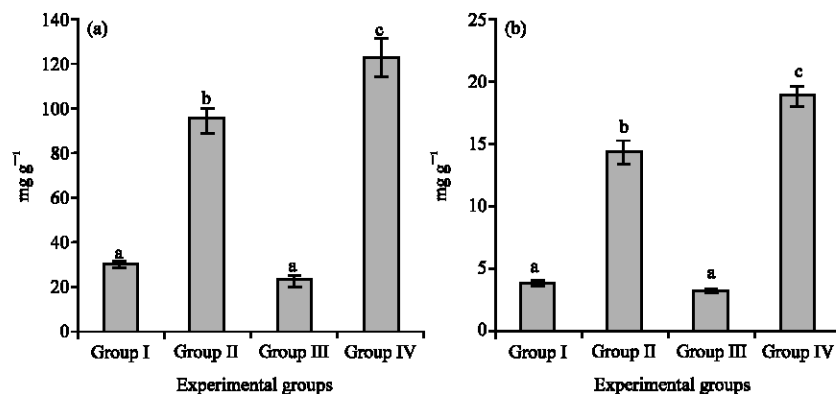


Fig. 1: (a) Level of total lipid content (mg g^{-1}) in the feces of control and experimental groups of rats and (b) level of total cholesterol content (mg g^{-1}) in the feces of control and experimental groups of rats. Group I animals were fed on standard diet with added coconut oil at 2% level for 45 days, group II animals were fed on standard diet with added 2% level squalene for 45 days, group III animals were fed on standard diet with added simvastatin at 20 mg for 45 days and group IV animals were fed on standard diet with added squalene (2%) and simvastatin (20 mg) for the same period. Results are Mean \pm SD for 6 animals. Samples followed by the same letter are not significantly different in Tukey's test using 0.05 level of significance

Groups IV rats that obtained the combined treatment of both squalene and simvastatin showed a significant increase in the excretion of cholesterol compared to both group I control rats as well as group II and group III treated rats (Fig. 1a, b), which indicates that the combination of both simvastatin and squalene is much more effective in eliminating cholesterol in feces than squalene or simvastatin alone. Even though squalene is a feedback inhibitor of HMG CoA reductase, it stimulates acylcoenzyme A: cholesterol acyltransferase (ACAT) (Standberg *et al.*, 1989). Simvastatin has been reported to alter the activity of intestinal ACAT in rabbits leading to reduced cholesterol absorption (Owens *et al.*, 1991). The combined effect of inhibition of ACAT by simvastatin and higher fecal cholesterol excretion by squalene might be the reason for the increased cholesterol excretion when they are used in combination.

Thus the results of the present study suggest that squalene a prime HMG CoA reductase inhibitor can also be used as a prudent dietary addition for individuals using similar cholesterol lowering drugs. However, further studies are needed for the precise evaluation of dietary squalene on cholesterol metabolism in humans as the pathway of hepatic bile acid synthesis in rats and mice are different from humans.

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REFERENCES

- Chan, P., B. Tomlinson, C.B. Lee and Y.S. Lee, 1996. Effectiveness and safety of low-dose pravastatin and squalene, alone and in combination in elderly patients with hypercholesterolemia. *J. Clin. Pharmacol.*, 36: 422-427.

- Farvin, K.H.S., R. Anandan, S.H.S. Kumar, K.S. Shiny, T.V. Sankar and T. K. Thankappan, 2004. Effect of squalene on tissue defence system in isoproterenol-induced myocardial infarction in rats. *Pharmacol. Res.*, 50: 231-236.
- Folch, J., M. Less and S.G.H. Sloane, 1957. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-509.
- Gregory, S. and N.D. Kelly, 1999. Squalene and its potential clinical uses. *Altern Med. Rev.*, 4: 29-36.
- Miettinen, T.A. and H. Vanhenen, 1994. Serum concentration and metabolism of cholesterol during rapeseed oil and squalene feeding. *Am. J. Clin. Nutr.*, 59: 356-363.
- Ohsawa, K., T. Watanabe, R. Matsukawa, Y. Yoshimura and K. Imaeda, 1984. The possible role of squalene and its peroxide of the sebum in the occurrence of sunburn and protection from the damage caused by U.V irradiation. *J. Toxicol. Sci.*, 9: 151-159.
- Owens, D., P. Collins, A. Johnson, O. Tighe, K. Robinson and G.H. Tomkin, 1991. Hypercholesterolaemia: simvastatin and pravastatin alter cholesterol metabolism by different mechanisms. *Biochim. Biophys. Acta.*, 1082: 303-309.
- Parekh, A.C. and D.H. Jung, 1970. Cholesterol determination with ferric acetate- uranyl acetate and sulphuric acid-ferrus sulphate reagents. *Anal. Chem.*, 42: 1423-1427.
- Qureshi, A.A., J.W. Lehman and D.M. Peterson, 1996. Amaranth and its oil inhibit cholesterol biosynthesis in 6 week old female chickens. *J. Nutr.*, 126: 1972-1978.
- Ryan, E., K. Galvin, T.P. O'Connor, A.R. Maguire and N.M. O'Brien, 2007. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains and legumes. *Plant Foods Human Nutr.*, 62: 85-91.
- Strandberg, T. E., R.S. Tilvis and T.A. Miettinen, 1989. Variations of hepatic cholesterol precursors during altered flows of endogenous and exogenous squalene in the rat. *Biochim. Biophys. Acta.*, 1001: 150-156.
- Strandberg, T.E., R.S. Tilvis and T.A. Miettinen, 1990. Metabolic variable of cholesterol during squalene feeding in humans comparison with cholesterolamine treatment. *J. Lipid Res.*, 31: 1637-1643.
- Thankappan, T.K., 2003. Isolation of Squalene from Shark Liver Oil. In: Society of Fisheries Technologists Seafood Safety, Surendran, P.K., Mathew, P.T. Thampuran, N. Nambiar, N. Joseph J. Boopendranath, P.T. Laksmanan, P.G.V. Nair (Eds.). Cochin, India, pp:173-175.
- Tilvis, R.S. and T.A. Miettinen, 1983. Dietary squalene increases tissue sterols and fecal bile acids in the rat. *Lipids*, 18: 32-36.