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SHORT COMMUNICATION

EFFECT OF 23-METHYL-21-NORCHOLEST-5-ENE-3β,23,25-TRIOL ON HEPATIC CHOLESTEROL 7α-HYDROXYLASE ACTIVITY IN THE RAT

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SUMMARY

The oxygenated sterol 23-methyl-21-norcholest-5-ene-3β,23,25-triol (SC-31082) has previously been reported to suppress 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity and to inhibit cholesterol ester accumulation. In view of these demonstrated activities and because of a lack of data concerning the effects of oxygenated sterols on other catabolic pathways for cholesterol, SC-31082 was evaluated for its effects on the rate-limiting enzyme in bile acid production, 7α-hydroxylase. The administration of SC-31082 to male rats (100 mg/kg) for 7 days resulted in a 2-fold increase of the specific activity of 7α-hydroxylase over that of control rats maintained under identical lighting conditions and this elevated activity was higher than that found in control rats at the peak of their circadian rhythm. Despite the sterol-induced elevated 7α-hydroxylase activity, there was no apparent reduction in either serum or hepatic microsomal cholesterol levels.

INTRODUCTION

A potential exists for sterols to regulate cholesterol metabolism at multiple points. Oxygenated sterols have recently been shown to suppress the synthesis of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) [1, 2, 3] and this suppression of HMG-CoA reductase by some sterols was accompanied by an inhibition of cholesterol ester formation [4]. Bile acids have been found to alter cholesterol catabolism or the enzymatic activity associated with the conversion of cholesterol to bile acids [5].

SC-31082 (23-methyl-21-norcholest-5-ene-3β,23,25-triol) was found to suppress HMG-CoA reductase and prevent cholesterol ester accumulation by inhibiting acyl-coenzyme A: cholesterol acyltransferase (EC 2.3.1.26) (ACAT) activity in cultured human fibroblasts [4]. Because of these demonstrated activities, and a lack of data concerning the effects of oxygenated sterols on enzymes associated with bile acid synthesis, we evaluated SC-31082 for its ability to affect 7α-hydroxylase activity *in vivo* and looked for associated changes in serum or hepatic microsomal cholesterol levels.

MATERIALS AND METHODS

[^{14}C]-Cholesterol with a S.A. of 50–60 mCi/mmol was obtained from New England Nuclear (Boston, Massachusetts). Polyoxyethylene Sorbitan Mono-oleate® (Tween 80) and NADPH were obtained from Sigma Chemical Company (St. Louis, Missouri). SC-31082 (23 methyl-21-norcholest-5-ene-3β,23,25-triol) was synthesized at Searle Laboratories (Skokie, Illinois).

Male adult Charles River rats (CRLi CD, Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts) weighing 370–450 g were used. Two groups of animals (6 animals each) were maintained on a standard light cycle with light from 7 a.m. to 7 p.m. and dark from 7 p.m. to 7 a.m. A third group (3 animals) was maintained on a reverse light cycle to demonstrate the elevated reductase activity seen in the dark cycle [6]. The two groups serving as controls received daily subcutaneous injections of olive oil for 7 days while the experimental group received daily injections of SC-31082 (100 mg/kg) with the compound suspended in olive oil. All rats were given standard laboratory rat chow (Purina Rat Chow) and water *ad libitum*. After 7 days, blood was drawn from the abdominal artery at 9:00 a.m. and analyzed for serum cholesterol levels [7]. Animals were sacrificed immediately after bleeding and their livers excised, homogenized and the microsomal fractions were saved for enzyme assay.

The 7α-hydroxylase activity was assayed according to the basic procedure of Björkhem and Danielsson[8]. The [^{14}C]-sterols were identified and quantitated by t.l.c. Unlabeled 7α-hydroxycholesterol and 7β-hydroxycholesterol served as standards. The plates were developed in an ethyl acetate–hexane system (4:1, V/V). The amount of [^{14}C]-7α-hydroxycholesterol present was measured by counting the regions on the t.l.c. plates corresponding to standard 7α-hydroxycholesterol by liquid scintillation spectrometry. Liver microsomal samples were also analyzed for cholesterol content [9] and protein content [10].

RESULTS AND DISCUSSION

The results of analyses of 7α-hydroxylase activity, serum cholesterol and microsomal liver cholesterol in animals treated subcutaneously with SC-31802 are compared with those from control animals in both the standard and reverse light cycle (Table 1). Animals treated with SC-31082 showed a statistically significant 2-fold increase in 7α-hydroxylase activity when compared to control animals maintained under similar lighting conditions and elevated enzyme activity over that found in the animals in reverse lighting. Serum and microsomal liver cholesterol were unchanged by SC-31082 when compared to the control animals in standard lighting. When compared to the reverse lighting group, serum cholesterol was also unchanged; microsomal liver cholesterol for the reverse cycle group was lower than that found in both control and SC-31082 treated groups on the standard light cycle. SC-31082 had no effect on body wt.

These results show that SC-31082 stimulates 7α-hydroxylase activity *in vivo* in addition to its previously reported effects on HMG-CoA reductase activity and cholesterol ester accumulation. Because the enzymatic conversion of cholesterol to bile acids is thought to be the major route for removal of exchangeable cholesterol from the

Table 1. Effects of subcutaneous treatment of SC-31082 (23-methyl-21-norcholest-5-ene-3β,23,25-triol) on hepatic 7α-hydroxylase, serum cholesterol and microsomal cholesterol

Subcutaneous treatment	Light cycle	No. of animals	Specific activity of hepatic 7α-hydroxylase (pmol/mg prot., means ± S.E.)	Serum cholesterol (mg/dl, mean ± S.E.)	Endogenous liver cholesterol (μg/mg microsomal prot., mean ± S.E.)
Vehicle	Standard	6	1.7 ± 0.1	52.7 ± 2.4	19.9 ± 1.0†
SC-31082 (100 mg/kg)	Standard	6	3.3 ± 0.4*	48.7 ± 1.0	19.3 ± 0.5
Vehicle	Reverse	3	2.3 ± 0.4	50.8 ± 2.7	14.3 ± 1.4

Animals were maintained in either a standard light cycle (light from 7:00 a.m.–7:00 p.m.) or a reverse light cycle (light from 3:00 p.m.–3:00 a.m.). All animals were treated subcutaneously for 7 days with either olive oil vehicle or 100 mg/kg SC-31082. Animals were killed at 9:00 a.m. The analytical methods used for the determination of 7α-hydroxylase activity, cholesterol and protein concentrations are described in the text. 7α-hydroxylase activity was calculated by the amount of [^{14}C]-cholesterol converted to [^{14}C]-7α-hydroxycholesterol per mg protein.

* Significantly different from standard light control group (Student's 2-tail *t*-test) [$P = 0.01$].

† Significantly different from reverse light cycle group (Student's 2-tail *t*-test) [$P = 0.03$].

body [11], and the rate-limiting step in this pathway is 7α-hydroxylation of cholesterol [12], it might be expected that stimulation of 7α-hydroxylase would lead to a decrease in the cholesterol pool utilized by 7α-hydroxylase, which, in the rat, is located in the microsomal fraction of liver homogenates [13]. Despite the elevated 7α-hydroxylase activity produced by SC-31082, there was no apparent reduction in the cholesterol content of the microsomal liver fraction. Our experience in observing elevated 7α-hydroxylase activity without a reduction in hepatic cholesterol is consistent with at least one other report showing elevated 7α-hydroxylase activity without altered levels of hepatic (or serum) cholesterol [14].

This communication represents another definition of the biological activity of an oxygenated sterol and is, to our knowledge, the first demonstration of an oxygenated sterol other than bile acid which can stimulate 7α-hydroxylase activity. While several potential sites for regulation have been proposed from studies *in vitro*, results *in vivo* have been mixed due to the apparent rapid metabolism of some of the oxygenated sterols [15]. SC-31082 can effect an increase in 7α-hydroxylase activity *in vivo* and thus, together with 25-hydroxycholesterol, 7-ketocholesterol and 3β-hydroxy-5α-cholest-8(14)-en-15-one [*cf.* 16], represents an example of the potential for regulation of cholesterol metabolism by oxygenated sterols.

REFERENCES

1. Bell J. J., Sargent T. E. and Watson J. A.: Inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in hepatoma tissue culture cells by pure cholesterol and several cholesterol derivatives. *J. biol. Chem.* **251** (1976) 1745–1758.
2. Brown M. S. and Goldstein J. L.: Suppression of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and inhibition of growth of human fibroblasts by 7-ketocholesterol. *J. biol. Chem.* **249** (1974) 7306–7314.
3. Chen H. W., Kandutsch A. A. and Waymouth C.: Inhibition of cell growth by oxygenated derivatives of cholesterol. *Nature* **251** (1974) 419–421.
4. Goldstein J. L., Faust J. R., Dygos J. H., Chorvat R. and Brown M. S.: Inhibition of the synthesis of cholesteryl esters in human fibroblasts by a synthetic derivative of 7-ketocholesterol and by progesterone. *Proc. natn. Acad. Sci.* **75** (1978) 1877–1881.
5. Shefer S., Hauser S., Lapar V. and Mosbach E. H.: Regulatory effects of sterols and bile acids on hepatic 3-hydroxy-3-methylglutaryl CoA reductase and cholesterol 7α-hydroxylase in the rat. *J. Lipid Res.* **14** (1973) 573–580.
6. Van Canfort J.: Circadian rhythm of cholesterol-7α-hydroxylase activity in genetically blind rats. *J. interdiscipl. Cycle Res.* **5** (1974) 89–94.
7. Levine J., Morgenstern S. and Vlastelica D.: Automation in Analytical Chemistry. *Technicon Symposia* (1968).
8. Björkhem I. R. and Danielsson H.: 7α-hydroxylation of exogenous and endogenous cholesterol in rat-liver microsomes. *Eur. J. Biochem.* **53** (1975) 63–70.
9. Kates M.: *Techniques in Lipidology*. American Elsevier, New York (1972) p. 360.
10. Lowry O. H., Rosebrough N. J., Farr D. L., Randall R. J.: Protein measurement with the folin phenol reagent. *J. biol. Chem.* **193** (1951) 265–275.
11. Siperstein M. D., Jayko M. E., Chaikoff I. L. and Dauben W. G.: Nature of metabolic products of C^{14}-cholesterol excreted in bile and feces. *Proc. Sci. Exp. Biol. Med.* **81** (1952) 720–724.
12. Danielsson H., Einarsson K. and Johansson G.: Effect of biliary drainage on individual reactions in the conversion of cholesterol to taurocholic acid. *Eur. J. Biochem.* **2** (1967) 44–49.
13. Mitropoulous K., Venkatesan S., Balasubramaniam S. and Peters T.: The submicrosomal localization of 3-hydroxy-3-methylglutaryl-CoA reductase, cholesterol 7α-hydroxylase and cholesterol in rat liver. *Eur. J. Biochem.* **82** (1978) 419–429.
14. Erickson S. A., Cooper A. D., Matsui R. and Gould G.: 7-keto cholesterol: its effects on hepatic cholesterogenesis and its hepatic metabolism *in vivo* and *in vitro*. *J. biol. Chem.* **252** (1977) 5186–5193.
15. Kandutsch A. A., Chen H. W. and Heinger H.: Biological activity of some oxygenated sterols. *Science* **201** (1978) 498–501.