Communications to the Editor

[Chem. Pharm. Bull. 32(8)3305—3308(1984)]

OXYGENATED STEROLS AS INHIBITORS OF ENZYMATIC CONVERSION OF DIHYDROLANOSTEROL INTO CHOLESTEROL

Yoshihiro Sato,*,[a] Yoshiko Sonoda,[a] Masuo Morisaki,[b] and Nobuo Ikekawa[b]
Kyoritsu College of Pharmacy,[a] Shibakoen 1-chome, Minato-ku, Tokyo 105, Japan and Department of Chemistry,[b] Tokyo Institute of Technology, Ōokayama 2-chome, Meguro-ku, Tokyo 152, Japan

Seven oxygenated sterols were tested for their effect on cholesterol biosynthesis from 24,25-dihydrolanosterol by rat hepatic subcellular 10000 x g supernatant fraction. The sterols (40 μM) exhibited considerable inhibitory effects on the synthesis of cholesterol from [24,25-^{3}H]-24,25-dihydrolanosterol (18 μM). 5α-Cholest-8(14)-en-3β-ol-15-one had the greatest effect (64% inhibition). The biological importance of the inhibitory properties of the sterols is discussed.

KEYWORDS——cholesterol biosynthesis; lanosterol; [24,25-^{3}H]-24,25-dihydrolanosterol; 22-hydroxycholesterol; 24,25-epoxycholesterol; 5α-cholest-8(14)-en-3β-ol-15-one

It has been established that certain oxygenated sterols, e.g. 25-hydroxycholesterol and 7-oxocholesterol are potent inhibitors of sterol synthesis and consequently are cytotoxic in various mammalian cells.[1] One of the targets of this action of oxygenated sterols is 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting enzyme in the sterol synthetic pathway which catalyzes the formation of mevalonic acid.[1] This is consistent with our recent finding that oxygenated sterols are not toxic to the silkworm Bombyx mori, which lack de novo sterol biosynthesis.[2] In addition to the inhibition of sterol synthesis, oxygenated sterols also affect human polymorphonuclear leucocyte chemotaxis,[3] echinocyte formation of red blood cells,[4] and platelet aggregation in plasma.[5] These effects are perhaps related to the insertion of oxygenated sterols into plasma membranes and the consequent derangement of the membrane properties and function.[6] Furthermore, oxygenated sterols reportedly possess angiotoxic properties and are suspected

of being atherogenic.[7]

In our continuing investigations of the biological effects of oxygenated sterols,[2,5] we have now examined the influence of these compounds on enzymatic conversion of 24,25-dihydrolanosterol into cholesterol. By the same method as described previously,[8] [24,25-^{3}H]-24,25-dihydrolanosterol was incubated with the rat liver homogenate S_{10} fraction in the presence of oxygenated sterol. The results are summarized in Table I. Essentially identical results were obtained on incubation with [24-^{3}H]-lanosterol.[9] It is clear that all the oxygenated sterols tested have a considerably potent inhibitory effect on cholesterol synthesis from dihydrolanosterol and lanosterol. 5α-Cholest-8(14)-en-3β-ol-15-one was found to be the most potent of the sterols examined. Schroepfer and his coworkers[10] reported that this ketone caused slight (12-15%) inhibition of the synthesis of digitonin-precipitable sterols from labelled acetate, but not from mevalonate, upon incubation with the S_{10} fraction of rat liver, even though this compound is an inhibitor of sterol synthesis in cultured cells and a potent hypocholesterolemic agent in intact animals. Our present results appear to be at variance with that reported.[10] The main differences in experimental conditions between the two studies were the substrate used (acetate or mevalonate *vs.* dihydrolanosterol), the concentration of the substrate and test compounds, and the method of preparation of the emulsions of the compounds.

In our previous paper[11] concerning the effects of oxygenated lanosterol analogs, we demonstrated that 7-oxo-24,25-dihydrolanosterol was an extremely potent inhibitor of cholesterol synthesis from lanosterol. However, in the present experiment 7-oxocholesterol was found to be only marginally inhibitory. This is another indication of the importance of the steroid nucleus structure in eliciting the inhibitory activity,[12] and is reminiscent of the reported differential effect of 14α-ethyl-5α-cholest-7-ene-3β,15α-diol and its desethyl analog.[10,13]

The data shown in Table I suggest that the inhibitory effect of oxygenated sterols is highly dependent on the configuration of the epoxide or the hydroxyl on the side chain: (24S)-24,25-epoxycholesterol was a much more potent inhibitor than the 24R-isomer, and (22S)-22-hydroxycholesterol is less potent than the 22R-isomer. It is intriguing to note that the configurational effect of the 22-hydroxyl group was also observed in the interaction with dipalmitoylphosphatidyl choline in liposome,[14] and in the ADP-induced platelet aggregation.[5] The remarkable inhibitory effect of (24S)-24,25-epoxycholesterol, a natural product of mammalian steroid biosynthesis by rat liver enzyme,[15] suggests that this compound is

Table I. Cholesterol Biosynthesis during Incubation of the S_{10} Fraction of Rat Liver Homogenate with [24,25-^{3}H]-24,25-Dihydrolanosterol in the Presence of Oxygenated Cholesterol Derivatives

Compound	24,25-Dihydrolanosterol fr. (%)	Cholesterol fr. (%)	Inhibition (%)
None (control)*	27.7	22.3	-------
5α-Cholest-8(14)-en-3β-ol-15-one	58.2	8.1	64
7-Oxocholesterol	39.3	16.7	25
24-Oxocholesterol	44.1	13.2	41
(24S)-24,25-Epoxycholesterol	53.1	10.3	54
(24R)-24,25-Epoxycholesterol	44.2	15.4	31
(22S)-22-Hydroxycholesterol	42.0	16.4	26
(22R)-22-Hydroxycholesterol	44.9	15.1	32

[24,25-^{3}H]-24,25-Dihydrolanosterol[16] (8.85×10^6 dpm; 42.6 μCi/μmol, 18 μM) was incubated with S_{10} fraction (21.0-22.5 mg protein/ml) at 37°C for 3 h. The incubation mixture contained, in a total volume of 5 ml, 4 ml of S_{10} fraction and cofactors as described previously.[8] Incubation was started by the addition of the substrate and the test compounds (40 μM) as an emulsion (0.1 ml) with Tween 80 (3 mg). After incubation, MeOH and KOH were added to a final concentration of 50% and 10%, respectively. The mixture was heated at 70°C for 1 h, then extracted with CH_2Cl_2. The extracts, which contain endogenous cholesterol (0.8-1.0 mg), were washed with water, dried over sodium sulfate and concentrated to a few milliliters. After addition of carrier 24,25-dihydrolanosterol (1.0 mg) to the solution, it was subjected to silica gel TLC with CH_2Cl_2 as the mobile phase and the radioactive 4,4-dimethyl sterol fraction and 4,4-demethyl sterol fraction were separated. Appropriate amounts of 24,25-dihydrolanosterol were added to the eluate of the 4,4-dimethyl sterol fraction and it was recrystallized several times to a constant specific activity. The 4,4-demethyl sterol fraction separated by silica gel TLC was isolated as the digitonin-precipitable sterols and counted with a liquid scintillation spectrometer (Tracor Analytic Mark III). The amount of cholesterol biosynthesis was determined from the radioactivity of the 4,4-demethyl sterol fraction (i.e., cholesterol fraction). Results are expressed as the percentage inhibition as follows: Percent inhibition of cholesterol synthesis = [(percent yield of cholesterol in control - percent yield in run with test compound)/percent yield in control] x 100. Each incubation was carried out in triplicate and the standard deviation of each value listed was less than 5 percent.

* [24,25-^{3}H]-24,25-Dihydrolanosterol was converted to 4α-methyl sterol (13%) and sterones (10%) in addition to cholesterol. The rate (22%) of conversion of 24,25-dihydrolanosterol to cholesterol is much heigher than that reported by Gibbons and his associates,[17] whereas the same group[18] demonstrated that mevalonic acid was converted to cholesterol in 49% yield. The main difference in experimental conditions is the method of addition of the substrates. Tween 80 was used to emulsify the sterol, since 24,25-dihydrolanosterol is insoluble in water.

a biological regulator of steroid synthesis.

The present experiment clearly indicates that HMG-CoA reductase is not the sole target enzyme of sterol synthesis inhibition by oxygenated sterols, but lanosterol demethylation and/or the subsequent transformation ultimately leading to cholesterol are also affected by these compounds. It may be considered that cholesterol synthesis in organisms is controlled by oxygenated sterols not only at the stage of HMG-CoA reductase but also after formation of the steroid nucleus. The inhibitory effects are strictly related to the chemical structures of the oxygenated sterols. A more systematic study may provide greater insight into the relation between the chemical structure and the inhibitory effect.

REFERENCES AND NOTES

1) A. A. Kandutch, H. W. Chen and H. J. Heiniger, Science, 201, 498 (1978); P. R. Montellano, J. P. Beck and G. Ourisson, Biochem. Biophys. Res. Commun., 90, 897 (1979); R. Defay, M. E. Astruc, S. Roussillon, B. Descomps and A. Cates de Paulet, ibid, 106, 362 (1982).
2) B. Ying, M. Morisaki and N. Ikekawa, Chem. Pharm. Bull., 32, 3003 (1984).
3) L. I. Gordon, J. Bass and S. Yachnin, Proc. Natl. Acad. Sci., 77, 4313 (1980).
4) R. C. Hsu, J. R. Kanofsky and S. Yachnin, Blood, 56, 109 (1980).
5) H. Shimada, T. Imada, T. Kikuchi, Y. Saito, M. Morisaki, N. Ikekawa and Y. Inada, Biochem. Inter., in press.
6) S. Yachnin, R. A. Streuli, L. I. Gordon and R. C. Hsu, Curr. Top. Hematol., 2, 245 (1979); U. H. Ugli, R. A. Streuli and E. Dubler, Biochemistry, 23, 148 (1984).
7) H. Imai, N. T. Werthessen, V. Subramanyam, P. W. LeQuesne, A. H. Soloway and M. Kanisawa, Science, 207, 651 (1980).
8) Y. Sato and Y. Sonoda, Chem. Pharm. Bull., 32, 1912 (1984).
9) The percentage inhibition in the conversion of [24-^{3}H]-lanosterol into cholesterol was 54, 38, 25 and 35% by (24S)-24,25-epoxycholesterol, (24R)-24,25-epoxycholesterol, (22S)-22-hydroxycholesterol and (22R)-22-hydroxycholesterol, respectively.
10) L. R. Miller, T. P. Pajewsky and G. J. Schroepfer Jr., J. Biol. Chem., 257, 2412 (1982).
11) Y. Sonoda and Y. Sato, Chem. Pharm. Bull., 31, 1698 (1983).
12) Y. Sato, Y. Sonoda, M. Morisaki and N. Ikekawa, Chem. Pharm. Bull., 31, 1765 (1983).
13) D. L. Raulston, T. N. Pajewski, L. K. Miller, B. W. Phillip, D. J. Shapiro and G. J. Schroepfer Jr., Biochem. Inter., 1, 113 (1980).
14) H. Hagiwara, T. Nagasaki, Y. Inada, Y. Saito, Y. Yasuda, H. Kojima, M. Morisaki and N. Ikekawa, Biochem. Inter., 5, 329 (1982).
15) J. A. Nelson, S. R. Steckbeck and T. A. Spencer, J. Am. Chem. Soc., 103, 6974 (1981).
16) [24,25-^{3}H]-24,25-Dihydrolanosterol was prepared by catalytic tritiation of lanosteryl acetate in the presence of 5% Pd-C at The Radiochemical Centre, Amersham, England, followed by alkaline hydrolysis.
17) K. A. Mitropoulos, G. F. Gibbons, C. M. Connel and R. A. Woods, Biochem. Biophys. Res. Commun., 71, 892 (1976).
18) G. F. Gibbons, K. A. Mitropoulos and C. R. Pullinger, Biochem. Biophys. Res. Commun., 69, 781 (1976).

(Received May 23, 1984)