Solanum Alkaloids as Inhibitors of Enzymatic Conversion of Dihydrolanosterol into Cholesterol

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The effects of several solanum steroidal alkaloids on cholesterol biosynthesis from 24,25-dihydrolanosterol by 10000 × g supernatant fluid of rat liver were examined. Solacongestidine (I), solafloridine (II) and solasodine (III) (40 μM each) exhibited considerable inhibitory effects (59, 51, 37% inhibition, respectively) on the synthesis of cholesterol from [24,25-3H]-24,25-dihydrolanosterol (18 μM). The biological importance of the inhibitory properties of the steroidal alkaloids is discussed.

Keywords—cholesterol biosynthesis; [24,25-3H]-24,25-dihydrolanosterol; rat hepatic subcellular S10 fraction; solacongestidine; solafloridine; solasodine; solanum steroidal alkaloid

One of the present authors, Sato, and his coworkers have shown that some lanosterol and cholesterol analogs and oxygenated sterols are potent inhibitors of the enzymatic conversion of lanosterol and 24,25-dihydrolanosterol into cholesterol.1-3 This finding prompted us to examine whether other steroidal alkaloids, some of which show potent antifungal activity against Candida albicans, Trichophyton rubrum, etc.,4 are also inhibitors of cholesterol biosynthesis. We found that solacongestidine (I) is a potent inhibitor of cholesterol biosynthesis from dihydrolanosterol, compared with the other solanum alkaloids and oxygenated sterols already reported by Sato et al.

Experimental

Solacongestidine (I), solafloridine (II), solasodine (III), tomatidine (IV), tomatillidine (V) and solanocapsine (VI) were obtained as a result of the research on solanum alkaloids in the Steroids Section of the National Institute of Arthritis, Metabolic and Digestive Disease (NIAMDD) of the National Institutes of Health (NIH) in the U.S.A. The substrate, [24,25-3H]dihydrolanosterol, was prepared as described previously.5 Experiments to examine the effects of solanum alkaloids on cholesterol biosynthesis from 18 μM [24,25-3H]dihydrolanosterol rat hepatic subcellular fraction (S10 fraction) were performed as described previously.5

Results and Discussion

In the previous studies,1,2) the substrate and test compounds were used at the concentrations of 18 and 40 μM, respectively, and the structure–activity relationship was examined. The studies demonstrated that both the side chain and skeleton of the test compounds are important for the inhibitory activity on cholesterol synthesis. In the present study, the influence of solacongestidine (I) and solafloridine (II) along with solasodine (III), tomatidine (IV), tomatillidine (V) and solanocapsine (VI) on enzymatic conversion of dihydrolanosterol into cholesterol was examined. By the same method as described previously,5) [24,25-3H]dihydrolanosterol (18 μM) was incubated with the rat liver homogenate
The results are summarized as Table I. It is clear that some of the alkaloids (I, II, III) inhibited cholesterol synthesis from dihydrolanosterol. Solacongestidine (I) was found to be the most potent, followed by solafloridine (II). The potency of I (59% inhibition) was almost the same as that of 3β-hydroxy-5α-cholest-8(14)-en-15-one (64%), one of the most potent oxygenated cholesterol derivatives, examined by the same method. It is of interest that solanum alkaloids such as solacongestidine (I) and solafloridine (II) are inhibitors of cholesterol biosynthesis from dihydrolanosterol. The recovery yield of the substrate, dihydrolanosterol, from the incubation medium with I and II was high (62.3 and 51.8%) compared with that from the control (26.0%). This suggests that one of the sites of inhibition by I and II is the step of 14α-demethylation of dihydrolanosterol.

Solacongestidine (I) and solafloridine (II) have been obtained as aglycones after hydrolysis of glycosides from Solanum congestiflorum. Both alkaloids, especially I, showed potent antifungal activity against Candida albicans, Trichophyton rubrum, Cryptococcus albidus, C. neoformans, Torulopsis candida and Trichosporon cutaneum, while other related compounds showed much lower activity. Neither biological nor pharmacological studies have been carried out on solacongestidine (I) except for our studies on the antifungal activity.
Therefore, it is noteworthy that I is a potent inhibitor of cholesterol biosynthesis from dihydrolanosterol. More systematic pharmacological and biological studies may provide greater insight into the biological activities of I and related compounds.

Ketoconazole, an N-substituted imidazole derivative, shows significant therapeutic effects upon some fungal diseases and it inhibits ergosterol synthesis in fungal cells with accumulation of 14α-methyl sterols, indicative of an interaction with the 14α-demethylase system.\(^7\) This reaction is carbon monoxide-sensitive, indicating that a cytochrome P-450-containing enzyme system is required to initiate oxidation of the 14α-methyl group of lanosterol and dihydrolanosterol.\(^8\) The accumulation of 14α-methyl sterols in fungal cells has been suggested to lead to functional changes in cell membranes and to cell death. The biosynthesis of cholesterol and ergosterol from lanosterol involves similar steps for the removal of the three methyl groups.\(^9\) It has been established in the case of ketoconazole that the dose required to influence cholesterol synthesis in rat liver is at least six times that required to inhibit ergosterol synthesis in Candida albicans.\(^{10}\) It has also been shown that cholesterol synthesis in a subcellular fraction of rat liver is about 20—27 times less sensitive to ketoconazole than ergosterol synthesis in a similar fraction obtained from C. albicans.\(^{10}\)

Solanagrostidine (40 µM) exhibited 59% inhibition of cholesterol synthesis from [24,25,\(^3\)H]dihydrolanosterol (18 µM). On the other hand, the minimum inhibitory concentration (MIC) of solanagrostidine (I) against C. albicans is less than 1 µg/ml (2.5 nm/ml). The influence of solanagrostidine (I) on ergosterol synthesis should be examined.

The structure of solanagrostidine (I) is similar to that of 25-azasteroids, e.g., 25-azacholesterol [24-dimethylamino-cholest-5-en-3β-ol]. This azasteroid and 25-azacoprostane [24-dimethylamino-5β-cholan] are highly active inhibitors of insect molting and metamorphosis,\(^{11}\) and they also show inhibitory effects on the growth and development of the free-living stages of nematodes such as Nippostrongylus brasiliensis and Nematospioirides dubius.\(^{12}\) These azasteroids, though their mode of inhibitory action is different from that of the compounds tested in the present studies, have been established to inhibit the Δ\(^{24}\)-sterol reductase enzyme system, resulting in the disturbance of essential sterol synthesis and the inhibition of growth of insects and nematodes, which lack de novo sterol biosynthesis and require exogenous sterols, utilizing sterols of both plant and animal origin.\(^{13,14}\) As judged from the above discussion, our finding that solanum alkaloids, especially solanagrostidine (I), are potent inhibitors of cholesterol biosynthesis from dihydrolanosterol suggests that some solanum alkaloids and related compounds may be biological modulators of sterol biosynthesis (anabolic and catabolic).

References and Notes