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MINIREVIEW

POLYOXYGENATED STEROLS AND TRITERPENES: CHEMICAL STRUCTURES AND BIOLOGICAL ACTIVITIES

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Summary—Cholesterol and other sterols are precursors of many hormones of vertebrates (pregnane, androstane, estrane derivatives), invertebrates (ecdysteroids) and even of plants (brassinoids). The normal biosynthetic processes which begin by a series of oxidations lead to a family of compounds, some of which exhibit a wide variety of biological activities. Among the latter, those well-established are their inhibitory effect on the biosynthesis of cholesterol in mammalian cells, their toxic effect on tumor cells and their ability to modify some immunological responses. None of the members of this family has all of the activities just mentioned. The intensity of the effect depends markedly upon the specific structure of each compound.

INTRODUCTION

Since the first report on the biological activities of oxygenated derivatives of cholesterol fifteen years ago [1], this class of chemical compounds has been found to exhibit many biological effects [2]. Among the latter, the most systematically and extensively studied are the inhibition of cholesterol biosynthesis in mammalian cells, the selective cytotoxicity towards tumor cells and the ability of several of them to modulate some immunological responses. Initially, the substances used by Kandutsch and Chen for the studies of the regulation of cholesterol biosynthesis were simple autoxidation compounds of cholesterol, such as cholesterol derivatives bearing a hydroxy or a keto group at C-7, C-20, C-22 or C-25, **1**. Since then, many sterols (including phytosterols) and tetracyclic triterpenes, bearing one or several different oxygenated functions have shown the biological effects mentioned above. They should be more precisely termed "polyoxygenated sterols and triterpenes", **2**.

They derive from the normal metabolism of cholesterol or its precursors. Cholesterol, which is a membrane structural constituent, can be transformed into numerous biological compounds, for example to cholic acid, corticosteroids, sex hormones and calciferol. In invertebrates, it gives rise to the moulting hormone ecdysone. The conversion of cholesterol into these physiologically active compounds entails the formation of a series of intermediates which are generated by enzymatic or chemical reactions. These intermediates also exercise biological functions as they are structurally related to the normal biological substrates. In this paper, we summarize the recent results on the inhibition of sterol biosynthesis induced by some of these oxygenated sterols. Special emphasis will be given to those which induce antitumor and immunomodulating effects.

INHIBITION OF CHOLESTEROL BIOSYNTHESIS

Several oxygenated sterols inhibit the biosynthesis of cholesterol in cultured cells efficiently by depressing the activity of HMGCoA reductase [3]. It appears that:

—the inhibitory effect increases as the distance between the hydroxy at C-3 and the second oxygenated group is increased: oxysterols bearing a hydroxy group in the side chain or in the D-ring are the most potent inhibitors,
—a complete side chain is necessary to induce high inhibition, when the side chain is shortened, the inhibitory effect decreases,
—to have a potent inhibitory effect, the second hydroxy group should be less hindered [4].

In living animals, some common oxygenated sterols are metabolized quickly and their biological effects are thus limited. Sterols bearing an oxygenated function in the D-ring (e.g. compound **3**) which are metabolized slowly, have a potent cholesterol lowering effect when administered to rodents and

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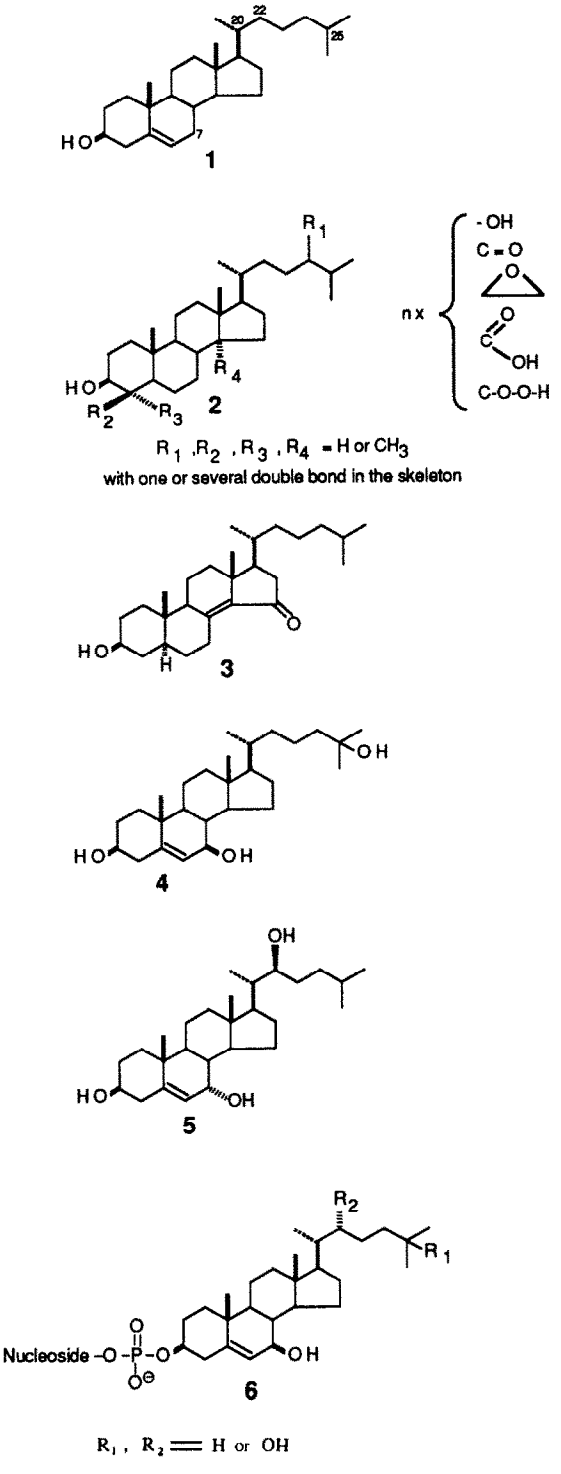

Scheme 1

nonhuman primates. Recent studies show that polar metabolites are responsible for such an effect [5].

Mechanistically, the activity of oxysterols on the inhibition of cholesterol biosynthesis seems to be that of a hormone-like effect. Moreover, some authors have provided evidence for the presence of specific oxysterol binding proteins [6, 7].

SELECTIVE CYTOTOXICITY AND ANTITUMOR EFFECT

In a research program designed to look for antitumor agents, we have shown that several sterols and triterpenes bearing one or several oxygenated functions at different positions of the sterol skeleton are toxic at 10^{-6} M towards tumor cells cultured *in vitro* [8]. They have no toxic effect towards non-tumor cells under the same conditions. This selective cytotoxicity has been observed in studies with murine hepatoma and lymphoma cells [9] and with human tumor cells.

This selective cytotoxicity depends markedly on the precise chemical structure of the sterols. 7β-hydroxycholesterol which is very toxic towards hepatoma cells is only moderately toxic towards lymphoma cells. On the other hand, 25-hydroxycholesterol which is very toxic towards lymphoma cells has no effect on hepatoma cells. These data have prompted us to synthesize 7β,25-dihydroxycholesterol **4** which has potent effects on both cellular systems. In an analogous manner, 22R-hydroxydesmosterol is very cytotoxic while 22S-hydroxydesmosterol is inactive. The combination of hydroxy groups at C-7 and at C-22 in the cholesterol skeleton lead to four stereoisomers, of which 7α,22S-dihydroxycholesterol **5** is by far the most active one. Up to now, no clear structure–activity relationship has been detected.

This selective cytotoxicity is in fact differential: in a mixed culture of cardiac cells, the fibroblasts are destroyed by 7β-hydroxycholesterol while the myocytes are not affected [10].

This cytotoxicity is characterized by a rapid lysis of the cells which disappear in culture media. The same phenomenon has also been observed *in vivo* [11].

The high lipophilicity of sterols makes *in vivo* studies with animals difficult. To overcome this inconvenience, water soluble prodrugs have been prepared. The sodium salt of bishemisuccinate of 7-hydroxycholesterol has a potent antitumor effect on mice bearing ascitic carcinoma Krebs 2 [11], leukemia L1210 and P388, sarcoma S180 and Ehrlich ascitic tumor. However, owing to its low water solubility (less than 2%) several *in vivo* studies are difficult to carry out. Therefore, we have recently synthesized phosphodiesters of oxygenated sterols **6** (more than 30% solubility in water) which are more appropriate for *in vivo* studies [12].

Some polyoxygenated sterols such as 7,22-dihydroxycholesterol are sufficiently water-soluble to be administered in drinking water. In this way, an interesting inhibitory effect was obtained on the DMBA-induced carcinoma in the rat [13].

Since it is antagonized by exogenous cholesterol, this cytotoxicity is presumably a consequence of the inhibition of cholesterol biosynthesis. Though many oxysterols are potent inhibitors of cholesterol biosynthesis in some cell lines, they are not toxic towards them. Thus, there is no close relationship between cytotoxicity and inhibition of cholesterol biosynthesis.

The cell lysis suggests that one of the targets of oxysterols is the cell membrane. This hypothesis is supported:

(a) by an ultrastructure investigation by means of the electron microscopy which showed an early alteration of cell membrane,
(b) by a fluorescent study which demonstrated an effect on membrane fluidity [14],
(c) by incorporation of radiolabelled oxysterols which proved that there is an interaction between oxysterols and cell membrane [15] and
(d) by an investigation of the metabolism of oxysterols [16].

IMMUNOMODULATING EFFECT OF OXYGENATED STEROLS. CONFIRMATION OF AN EFFECT AT AN EARLY STEP OF LYMPHOCYTE ACTIVATION

Oxygenated sterols are not toxic towards normal lymphocytes, but they inhibit the blastogenesis of lymphocytes when they are stimulated by mitogens or antigens [9, 17]. A structure–activity relationship has been established [18]. It is similar to that for the inhibition of sterol biosynthesis [4].

This immunosuppressive effect induces the inhibition of both interleukin-2 production and the appearance of Il-2 receptor [19].

It can be partially reversed by foetal calf serum, purified LDL and high concentrations of K^+ [20]. The fact that K^+ is able to antagonize the 7β-hydroxycholesterol immunosuppression provides further evidence for the cell membrane being implicated in the oxysterol effect.

Oxygenated sterols inhibit the stimulation of lymphocytes by ionomycin and phorbol myristate acetate [21]. Thus, they do not affect Ca^{2+} mobilization. Our preliminary results show a marked effect of 7,25-hydroxycholesterol on PKC activity (to be published).

From all these results, it is clear that the cell membrane may be a target of oxygenated sterols. This fact is very important as it has been suggested that investigation of anticancer agents acting at this level may initiate a new approach to drug research in cancer chemotherapy [22].

We are in the presence of a family of compounds which demonstrate a wide variety of biological effects. However, none of these compounds possesses all of the biological activities exhibited by the family. The intensity of each biological effect depends markedly on the precise chemical structure of each compound.

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