Neuroscience Letters 525 (2012) 44–48

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Protective effects of 27- and 24-hydroxycholesterol against staurosporine-induced cell death in undifferentiated neuroblastoma SH-SY5Y cells

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HIGHLIGHTS

- Effects of 27-hydroxycholesterol (27OH) and 24-hydroxycholesterol (24OH) were studied.
- 27OH counteracted the toxic effect of staurosporine on neuroblastoma SH-SY5Y cells.
- 27OH counteracted staurosporine-mediated induction of caspases involved in apoptosis.
- 24OH had similar effects as 27OH in low concentrations but opposite in high ones.
- The present data indicate that oxysterols can have neuroprotective effects.

ARTICLE INFO

Article history:
Received 8 May 2012
Received in revised form 25 July 2012
Accepted 26 July 2012

Keywords:
Oxysterol
Cholesterol metabolism
Cell viability
Neuroprotection
Neurodegeneration
Apoptosis

ABSTRACT

Alterations in cholesterol metabolism have been linked to several neurodegenerative disorders, including Alzheimer's disease, multiple sclerosis and Parkinson's disease. Brain cholesterol is metabolized to the oxysterols 24-hydroxycholesterol and 27-hydroxycholesterol. Disturbed levels of these oxysterols are found in neurodegenerative conditions. In the current study we examined the effects of 27- and 24-hydroxycholesterol on viability of human neuroblastoma SH-SY5Y cells treated with staurosporine, a toxic substance that induces apoptosis. Analyses using MTT assay and measurement of lactate dehydrogenase release showed that presence of 27-hydroxycholesterol counteracted the toxic effects of staurosporine on these cells. Also, 27-hydroxycholesterol significantly decreased the staurosporine-mediated induction of caspase-3 and -7, known to be important in apoptotic events. 24-Hydroxycholesterol had similar effects on viability as 27-hydroxycholesterol in low concentrations, although in higher concentrations this oxysterol exacerbated the toxic effects of staurosporine. From these findings it may be concluded that effects of oxysterols on cellular viability are strongly dependent on the concentration and on the type of oxysterol. Previous studies on oxysterols have reported that these compounds are pro-apoptotic or trigger pathological changes that result in neurodegeneration. The present data indicate that, during some conditions, oxysterols may have neuroprotective effects.

1. Introduction

Cholesterol is vital for brain development and function. The maintenance of cholesterol homeostasis is therefore very important in this organ [7,18]. Alterations in cholesterol turnover have been linked to several neurodegenerative disorders, including Alzheimer's disease (AD), multiple sclerosis and Parkinson's disease [1,7,18]. Brain cholesterol is metabolized to the less hydrophobic 24- and 27-hydroxycholesterol (Fig. 1), which can pass the brain–blood barrier [1,13]. Disturbed levels of these oxysterols (hydroxylated derivatives of cholesterol) have been observed in several neurodegenerative conditions [7,18]. 24-Hydroxycholesterol is formed exclusively in the CNS whereas 27-hydroxycholesterol is formed in many different tissues. This compound, which is found in high amounts in cells of the vasculature, can pass efficiently to the CNS from the circulation [6,17]. 24-Hydroxycholesterol and 27-hydroxycholesterol are believed to play important roles as regulators of cholesterol homeostasis and elimination [1,13]. However, high amounts of these and other oxysterols are known to be cytotoxic to a number of cell types in the CNS and elsewhere [4,9,19].

Abbreviations: AD, Alzheimer's disease; CNS, central nervous system; CSF, cerebrospinal fluid; CYP, cytochrome P450; GC–MS, gas chromatography–mass spectrometry; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide.

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http://dx.doi.org/10.1016/j.neulet.2012.07.057

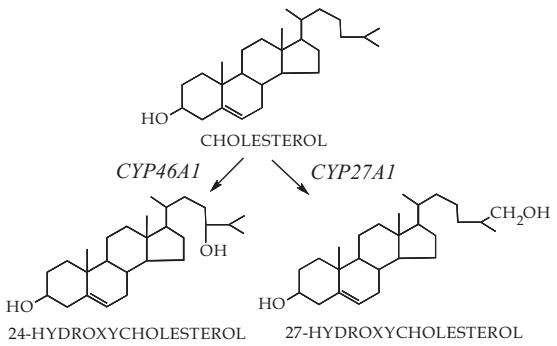

Fig. 1. Chemical structures of 24-hydroxycholesterol and 27-hydroxycholesterol formed by the enzymes CYP46A1 and CYP27A1, members of the cytochrome P450 (CYP) family. For more information on these enzymes, see Refs. [1,13].

As mentioned above, oxysterols are important for regulation of cholesterol homeostasis in the CNS. 24-Hydroxycholesterol may also have a positive impact with regard to brain pathology. This oxysterol is reported to inhibit formation of β-amyloid, an important component in the development of AD [3,7]. Nevertheless, a large number of studies report harmful effects of oxysterols on tissues or cells from the CNS [5,9,16,19]. Oxysterols have been found to be pro-apoptotic, induce necrosis or neuroinflammation, or trigger pathological changes that result in neurodegeneration. Oxysterols also negatively impact the viability of cell types other than those of the CNS, including retinal pigment epithelial cells [4].

In the current study, we investigated the effects of 27- and 24-hydroxycholesterol on human neuroblastoma cells treated with staurosporine, a toxic model substance used to induce apoptosis [8]. Our findings indicate that, during some conditions, oxysterols may have neuroprotective effects.

2. Materials and methods

2.1. Materials

27-Hydroxycholesterol, prepared from kryptogenin was a kind gift from Dr. L. Tökes, Syntex, Palo Alto, CA, USA. 24-Hydroxycholesterol was synthesized as described [12]. The purity of the oxysterols was >95% as determined by GC–MS (gas chromatography mass spectrometry). The human neuroblastoma cell line SH-SY5Y (ATCC CRL-2266) was obtained from the American Type Culture Collection (Rockville, MD, USA). Materials for cell culturing were obtained from Gibco. Staurosporine and MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) were purchased from Sigma.

2.2. Cell cultivation and treatment with staurosporine

Human undifferentiated neuroblastoma SH-SY5Y cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with the addition of 10% fetal bovine serum and 1% antibiotic–antimycotic. This cell line, which may be used as a model for neuronal cells [2,11,14], is derived from a human tumor (neuroblastoma) and considered most similar to an immature cell of the sympathetic nervous system. Prior to experiments, the cells were seeded in 96-well plates with approximately 2×10^4 cells/well and left overnight. The next day medium was changed to DMEM without phenol red, supplemented with 2% charcoal-stripped fetal bovine serum. Cells were treated with oxysterols (10 nM to 20 μM) dissolved in ethanol, followed by treatment with staurosporine. Cells treated with vehicle (ethanol) were used as controls. Samples of cells were counted before and after oxysterol treatment to examine if this affected the cell number. Neither 24- nor 27-hydroxycholesterol significantly altered the cell count, indicating that the oxysterols do not affect growth rate under these conditions.

Staurosporine, a microbial alkaloid, has been used in many studies to induce apoptosis. Staurosporine may also influence proliferation and differentiation [15]. This compound affects several signaling pathways. A well-known target for staurosporine is protein kinase C. The main biological activity of staurosporine is the inhibition of protein kinases through the prevention of ATP binding to the kinase.

SH-SY5Y cells were treated with 30–80 nM of staurosporine. Different concentrations were used in different sets of experiments because the quantitative response to staurosporine varied somewhat depending on the batch of staurosporine and on the cell passage number. With too few remaining live cells, the oxysterols were not able to compensate for the effects by staurosporine. Thus, the staurosporine concentration in each experiment was adjusted to a level resulting in significant but not extreme decrease in viability.

2.3. Measurement of cell viability by MTT assay

Cell cultures were treated for 24 h with staurosporine and analyzed using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay. This method is based on the conversion of MTT to water-insoluble MTT-formazan by mitochondrial dehydrogenases of living cells, providing a quantitative measure of viability. Although often used to assay cytotoxicity, MTT assay is not specific for viability but may also reflect effects on proliferation. Steroids were added 2 h prior to the addition of staurosporine. Following exposure to staurosporine, the cells were incubated for 1 h with 1.8 mM of MTT, dissolved in phosphate-buffered saline. Medium was then removed and the purple formazan product formed from the reduction of tetrazolium salt in living cells was dissolved with isopropanol containing 0.7% SDS. The absorbance was measured at 570 nm using a microplate reader (POLARstar Optima BMG Labtech). Results are expressed as percentage of the absorbance in controls.

2.4. Measurement of LDH release

Cell cultures were treated for 24 h with staurosporine and lactate dehydrogenase (LDH) release into the cell media was measured using the Cytotoxicity Detection Kit^{PLUS} (Roche Diagnostics GmbH). Steroid was added 2 h prior to the addition of staurosporine. Dead cells or cells with disrupted plasma membranes show increased LDH enzyme activity in the culture supernatant which correlates with the formation of formazan. Analysis was carried out according to the manufacturer's recommendations and the absorbance was measured at 450 nm using a microplate reader. Results are expressed as percentage of the absorbance in the controls.

2.5. Measurement of caspase activity

Staurosporine-induced activity of caspase-3 and -7 was measured using the Caspase-Glo® 3/7 Assay kit (Promega). Cleavage of the luminogenic substrate results in a luminescent signal proportional to the caspase activity. 27-Hydroxycholesterol, dissolved in ethanol, was added to cells 24 h before addition of staurosporine. The cells were then treated with staurosporine for 4 h and the caspase activity was analyzed according to the manufacturer's recommendations. The luminescence was measured using a TD-20/20 luminometer (Turner Designs).