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Cancer Letters 228 (2005) 105–110
CANCER Letters
www.elsevier.com/locate/canlet

The role of gangliosides in fenretinide-induced apoptosis of neuroblastoma

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Received 6 December 2004; accepted 12 January 2005

Abstract

Fenretinide is thought to induce apoptosis via increases in ceramide levels but the mechanisms of ceramide generation and the link between ceramide and subsequent apoptosis in neuroblastoma cells is unclear. In SH-SY5Y neuroblastoma cells, evidence suggests that acid sphingomyelinase activity is essential for the induction of ceramide and apoptosis in response to fenretinide. Downstream of ceramide, apoptosis in response to fenretinide is mediated by increased glucosylceramide synthase activity resulting in increased levels of gangliosides GD3 and GD2 via GD3 synthase. GD3 is a key signalling intermediate leading to apoptosis via the activation of 12-Lipoxygenase, and the parallel induction of GD2 suggests that fenretinide might enhance the response of neuroblastoma to therapy with anti-GD2 antibodies.

Keywords: Apoptosis; Fenretinide; Gangliosides; Ceramide; GD2; GD3

1. Introduction

The most significant advance in treatment for neuroblastoma in recent years has come from the inclusion of 13-*cis* retinoic acid as a biological therapy targeting minimal residual disease [1]. Despite the well-known property of 13-*cis* retinoic acid as an inducer of neuroblastoma differentiation in vitro, most likely as a result of isomerisation to all-*trans* retinoic acid [2], there is considerable uncertainty about the mechanism of its efficacy in vivo. A recent study has suggested that 13-*cis* retinoic acid may sensitize neuroblastoma to cytotoxic lymphocytes and this might be an important mechanism of response to retinoid therapy [3]. Nevertheless, further refinements of treatment with retinoic acid may be limited by the increased resistance of retinoic acid-differentiated cells to chemotherapeutic drugs [4]. However, some synthetic derivatives of retinoic acid such as fenretinide [*N*-(4-hydroxyphenyl)

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doi:10.1016/j.canlet.2005.01.044

retinamide], a synthetic analogue of retinoic acid, are able to induce apoptosis rather than differentiation [5,6], and may be useful additional drugs for neuroblastoma therapy. Fenretinide is clinically well tolerated and is currently being used in clinical trials for neuroblastoma and other cancers (for example, see [7,8]). A significant factor in its development for neuroblastoma therapy is that, unlike 13-*cis* retinoic acid, fenretinide shows synergistic responses with chemotherapeutic drugs in vitro [9,10].

Using SH-SY5Y neuroblastoma cells as a model system, we have shown that fenretinide induces apoptosis of SH-SY5Y neuroblastoma cells via caspase-dependent signalling events involving mitochondria [11]. The inhibition of apoptosis by retinoic acid receptor (RAR) antagonists and antioxidants suggests that RARs and reactive oxygen species (ROS) are both required for fenretinide-induced apoptosis [11]. However, the induction of ROS or oxidative stress may be the critical factor underlying the ability of fenretinide to promote apoptosis. Current evidence suggests that oxidative stress induced by fenretinide in SH-SY5Y and HTLA230 neuroblastoma cells is mediated by 12-lipoxygenase (12-LOX) activity [12,13]. Downstream of 12-LOX, a sustained induction of the growth and DNA damage (GADD)-inducible transcription factor GADD153 [12] is a consequence of increased ROS [12], and subsequent induction of the pro-apoptotic protein Bak [14] accompanies mitochondrial cytochrome *c* release and caspase-3-dependent apoptosis [11]. Signalling events downstream of ROS are also mediated by NF-κB activity [15].

2. Ceramide in fenretinide-induced apoptosis

In many tumour cell types, fenretinide increases intracellular levels of the lipid secondary messenger ceramide [6,16–18] by activation of serine palmitoyltransferase, a rate-limiting enzyme in the de novo ceramide synthesis pathway [16–19]. However, intracellular ceramide levels can also increase as a result of hydrolysis of membrane sphingomyelin by neutral and acid sphingomyelinases (Fig. 1) [20,21]. Both these sphingomyelinase classes can be important in ceramide generation and apoptosis [22–26].

As in other cell lines [6,27], intracellular ceramide levels increase during fenretinide-induced apoptosis of SH-SY5Y cells [28]. In these cells, we have been unable to block fenretinide-induced apoptosis using fumonisin B1 [28], an inhibitor of ceramide synthase. Fumonisin B_1 is also relatively ineffective at blocking fenretinide-induced apoptosis of CHLA-90 and SK-N-RA neuroblastoma cells [16]. In contrast, inhibitors of sphingomyelinase are effective in blocking both apoptosis and fenretinide-induced ROS in SH-SY5Y cells and HTLA230 cells [28]. Desipramine, a specific inhibitor of neutral sphingomyelinase, does not block fenretinide-induced apoptosis in these cells, and this implicates acid sphingomyelinase as the sphingomyelinase involved in ceramide generation in response to fenretinide. This conclusion is supported by experiments in which acid sphingomyelinase expression has been reduced using small-interfering RNAs (siRNA), resulting in an inhibition of fenretinide-induced apoptosis, ROS and ceramide production [28]. Thus, the evidence for SH-SY5Y and HTLA230 cells suggests that the increase in ceramide in response to fenretinide treatment is derived from sphingomyelin via acid sphingomyelinase activity. Nevertheless, the relative contributions of de novo synthesis and sphingolipid hydrolysis to fenretinide-induced increases in intracellular ceramide levels may vary between different neuroblastoma cell lines. Fenretinide also induces necrosis when used at high concentrations and it is possible that, under these conditions, the de novo synthesis and sphingomyelinase pathways are both activated, but relate to different cell-death signalling pathways.

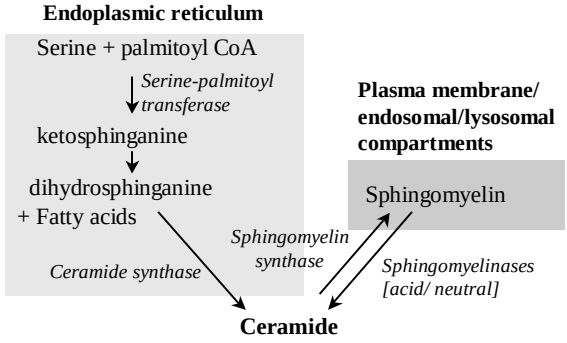

Fig. 1. Main pathways of Ceramide generation.