SciMedCentral

Journal of Pharmacology & Clinical Toxicology

Review Article

The New Age for Discovering New Selective Antitumor Agents: Antineoplastic Phospholipids

Lisley I. Mambelli[1]*, Salomão D. Jorge[1,2], Kerly Pasqualoto[2], Marcio Henrique Zaim[2,3], Ricardo A. de Azevedo[1,2], and Adilson Kleber Ferreira[1,2]*

[1]*Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil*

[2]*Alchemy, Innovation, Research & Development, University of Sao Paulo, Sao Paulo, SP, Brazil*

[3]*Institute of Chemistry, University of Sao Paulo, Sao Paulo, SP' Brazil*

***Corresponding author**

Lisley I. Mambelli, Department of Immunology, University of Sao Paulo, Brazil, Email: lis_mambelli@yahoo.com.br

Submitted: 23 February 2017

Accepted: 17 April 2017

Published: 19 April 2017

ISSN: 2333-7079

Keywords

- Antineoplastic phospholipids
- CTP: Phosphocholinecytidylyltransferase enzyme
- Kennedy's pathway; Apoptosis

Abstract

Cancer is responsible for millions of deaths worldwide. The relative lack of effective treatments for cancer makes extremely urgent the development of more efficient and selective new drugs. In contrast to the major part of commonly used chemotherapeutic drugs, the antineoplastic phospholipids (ALP) do not have DNA as a target. Such phospholipids are responsible for modifying the cell membrane turnover, inducing cell death by apoptosis, with high selectivity for tumor cells. The central hypothesis is that the antitumor effects of ALP are due to the inhibition of CTP: phosphocholinecytidylyltransferase (Pcyt-1) enzyme in the phospholipid biosynthetic pathway, known as Kennedy's pathway. The inhibition of the Pcyt-1 blocks the synthesis of phosphatidylcholine and causes the accumulation of ceramide, commonly associated with apoptosis. Moreover, important substrates, which are produced by this pathway are compromised and blocks the cell cycle progression, and consequently the correct cytokinesis. Mainly of them are also responsible for inducing a topological impairment of the trans-membrane protein domains, mostly located in mitochondria. Such fact affects the cellular bioenergetics, eventually triggering death by apoptosis. Besides, it impairs lipids signaling, which are directly associated with the regulation of mitogen-activated protein kinase (MAPKs) responsible for mediating signaling pathways. Therefore, the inhibition of Kennedy's pathway constitutes a promising strategy for the identification of novel antitumor compounds, and Pcyt-1, which is a key enzyme of such signaling/pathway, becomes the main potential target, with innovative character, for the development of antitumor drugs.

INTRODUCTION

New approaches for the development of antineoplastic chemotherapy

In the last 20 years, it has been observed a substantial change in the investigation of new targets for cancer treatment. The combination of new methodologies with the traditional ones, mainly taking the integration of specialists from different areas may contribute to the development of new approaches. Such interdisciplinary to study particularly potential molecular targets in tumor cellshas been in focus [1]. Of interest, these studiesincreasethe chances of finding new selective antitumor drugs, with the possibility of eliminating secondary cytotoxic effects, which are commonly associated with conventional chemotherapy. Similar to other therapeutic areas, the success of discovering new innovative molecules depends on the creative interaction between chemistry and biology.

The main process fornew drug discovery in cancer is based on the generation of the new hypothesis concerning the targets, which will give rise to the prototypes. Once found a new prototype; it is applied theoretical approaches *in silico*, which may drive the chemical synthesis of the molecule. In turn, it allows the validation of the biological hypothesis by experimental trials [2].

The development of antineoplastic phospholipids (ALP)

One suitable rationale of lysophospholipids as immunomodulators is based on their well-known biological activity as regulators of different cellular enzymatic activities;the activation of peritoneal macrophages and consequentlythe regulation of the immune response [3].

In 1960, Fischer, Westphal, and Munder, who were located in Freiburg, Germany, found that the accelerated and continuous conversion of lecithin to lysophosphatidylcholine (lysoPC) occurred in response to a mixture of substances containing lipases and phospholipases. This response was the first observation that is possible to obtain a synthetic lysoPC. Since this finding, the lysoPC has been investigated *in vivo* and *in vitro* as prototypes for drug discovery in the cancer field. Of note, there are several important studies investigating the role of lysoPC on

the immune system modulation. So far, the results obtained from these experiments confirmed the initial hypothesis that lysoPC possess a high immunomodulatory activity, which takes part of the humoral response and cellular response, as well [4].

The first analog of lysoPC was synthesized in the late 1960s by Eibl, Arnold, Weltzien and Westphal to increase the chemical stability of these compounds, enhancing their immunomodulatory capacity[5]. The structural modifications performed in the lysoPC, such as the substitution of the ester bond by the ether in the region that connects the glycerol to the hydrocarbon chain, have made the analogous structures resistant to the enzymes acetyltransferase and lysophospholipase [6]. In their study, Munder and colleagues demonstrated, for the first time, that some of the synthesized analogues, in particular, the group of derived alkyl ethers, showed cytotoxic and cytostatic activities in tumor cell lines. Such datagive rise to the study and the development of these compounds as a new class of antitumor agents [7,8].

Currently, synthetic antitumor lipids are divided into two subtypes: alkyl ether phospholipids and alkylphosphocholine derivatives. The first group is composedof the derivatives of alkyl ether phospholipid, named as ethers-lipids or alkylphospholipids. The structure of such compounds contains linkages between glycerol and hydrocarbons of the phospholipids. Like the main prototype of this class, we point out the 1-octadecyl-2-methylphosphorylcholine (ET-18-OCH3), known as edelfosine. It is important to highlight that edelfosine is a template for the generation of new ethers-derivatives.

Thus, the main application of edelfosine in clinical practice is for the treatment of acute leukemias, both myeloid and lymphoid. In particular, edelfosine provokes the apoptosis of leukemic cells [9,10]. Studiesconsidering the relation between chemical structure and cytotoxic activity have revealed that the anti-proliferative and apoptotic effects may be attributed to the long alkyl chain, at the sn-1 or sn-3 positions, consisting of at least 16 carbon atoms [11].

In the late 1980s, Eibl and Unger in Göttingen, Germany identified a new ether-lysophospholipid group called hexadecylphosphocholine (known as miltefosine), representative of the second subtype of synthetic antitumor lipids, derived from alkylphosphocholine. Moreover, miltefosine is structurally formed by a chain (ether-like bond) of 16-carbon attached to a phosphocholine molecule [5,12,13]. Accordingly, miltefosine showed potent cytotoxic activity *in vitro* against several tumor cell lines [14]. Unfortunately, miltefosine has a high hemolytic activity when applied intravenously. Thus, the clinical use of miltefosine is limited to topical application cutaneous metastases of the breast cancer and for cutaneous lymphoma. Of note, miltefosine is also used for the treatment of tropical leishmaniasis [15, 16].

The action mechanisms of antineoplastic phospholipids

The main mechanism of action of antineoplastic phospholipids differs from the most current chemotherapy drugs.Once they do not target the DNA or the cell cytoskeleton, but they act by modifying the *turnover* in the cell membrane [17]. This modification induces cell death by apoptosis with high selectivity for tumor cells, which makes these antineoplastic phospholipids promising for the development of new prototypes and analogues [18].

Although thisselect mechanism of action of antineoplastic phospholipids on tumor cells is not entirely understood, the main hypothesis is based on the high lipid fluidity of tumor cell membrane. This increase of membrane fluidity occurs through the alteration in the lipid metabolism of tumor cells. It, in turn, result in the reduction of total cholesterol, sphingomyelin deficiency and an increase in the percentage of unsaturated fatty acids in the neoplastic cell membrane [19]. Accordingly, these alterations would facilitate the insertion, with high selectivity, of the long non-polar chain of the hydrocarbon of the antineoplastic phospholipids into the membrane of the tumor cells. In contrast, normal cells, including fibroblasts, endothelial cells, polymorph nuclear neutrophils, lymphocytes and bone marrow progenitor cells would be relatively resistant to this insertion. Finally, it would, therefore, give them resistance to the cytotoxic effects of antineoplastic phospholipids [11,20].

When the antineoplastic phospholipids are inserted into the membrane, they interfere in the *turnover* of membrane phospholipids, thus affecting the generation of second messengers such as phosphatidic acid, diacylglycerol, and phosphoinositide. Also, they are also able to alter the physical properties of cell membranes. Such changes would be responsible for cell stress, which would eventually induce apoptosis [21].

On the other hand, the region of the cell membrane where the antineoplastic phospholipids insertion occurs may also influence their cytotoxic effect. The subdomains of the plasma membrane, which contain high concentrations of cholesterol and glycosphingolipids, named as lipid rafts, are regions thatantineoplastic phospholipidscan be accumulated. Throughout the recent work, it has been reported that antineoplastic phospholipids induce antitumor effects forming lipid rafts with Fas (CD95 or APO-1), a subgroup of tumor necrosis factor superfamily (TNF) receptors capable of initiating cell death signaling [19,22].

PERSPECTIVES

Most studies attribute the induction of apoptosis by antineoplastic phospholipids to their ability to inhibit phosphatidylcholine synthesis. Following this hypothesis, the molecular target of these antineoplastic phospholipids would be the key enzyme in the biosynthesis, Pcyt-1. This enzyme is located both in the nucleus and in the cytoplasm and acts as a limiting factor in the regulation of this phospholipid biosynthesis.

The reaction of phospholipid biosynthesisis mediated by an amino alcohol phosphotransferase, which uses cytidine diphosphocholine (CDP-choline) to synthesize phosphatidylcholine or cytidine diphosphoethanolamine (CDP-ethanolamine) for the synthesis of phosphatidylethanolamine. This last step of the synthesis is called *de novo* biosynthesis of phosphatidylcholine, a biosynthetic pathway classified as Kennedy's pathway [23].Thus, the block of Pcyt-1 by the antineoplastic phospholipids would lead to the inhibition of

phosphatidylcholine synthesis, and consequently also to the reduction of the sphingomyelin and diacylglycerol synthesis. Once this reduction of activity occurs, there would be the accumulation of ceramide, a molecule commonly associated with the induction of apoptosis [24].

The rational planning and the development of novel antitumor phospholipid derivatives could lead to the discovery of highly specific and efficient compounds with less adverse effects for further use as drug candidates in clinical trials. This new approach would make it possible to improve the life expectancy of patients with cancer.

REFERENCES

1. Burgess DJ. Tumour immunology: Context is key for TSLP, Nature Reviews Cancer. 2012; 12: 796-797.
2. Boyd MR, Paull KD. Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen, Drug Development Research. 1995; 34: 91-109.
3. Burdzy K, Munder P, Fischer H, Westphal O. Increase in the phagocytosis of peritoneal macrophages by lysolecithin, Zeitschrift fur Naturforschung. Teil B, Chemie, Biochemie, Biophysik, Biologie und verwandte Gebiete. 1964; 19: 1118.
4. Munder P, Modolell M, Ferber E, Fischer H. Phospholipids in quartz-damaged macrophages, Biochemische Zeitschrift. 1966; 344: 310.
5. Berkovic D. Cytotoxic Etherphospholipid Analogues, General Pharmacology: The Vascular System. 1998; 31: 511-517.
6. Eibl H, Arnold D, Weltzien HU, Westphal O. [On the synthesis of alpha and beta lecithins and their ether analogs], Justus Liebigs Annalen der Chemie. 1967; 709: 226-230.
7. Munder PG , Modolell M, Raetz W, Luckenbach GA. Primary antibody formation in vitro by mouse cells in a complete homologous system, Eur J Imunol. 1973; 3: 454-457.
8. Andreesen R, Modolell M, Weltzien HU, Eibl H, Common HH, Löhr GW et al. Selective destruction of human leukemic cells by alkyl-lysophospholipids, Cancer research. 1978; 38: 3894-3899.
9. Vogler WR, Shoji M, Hayzer DJ, Xie Y, Renshaw M. The effect of edelfosine on CTP: cholinephosphate cytidylyltransferase activity in leukemic cell lines, Leukemia research. 1996; 20: 947-951.
10. Mollinedo F, de la Iglesia-Vicente J, Gajate C, de Mendoza AEH, Villa-Pulgarin JA, de Frias M, et al. In vitro and in vivo selective antitumor activity of Edelfosine against mantle cell lymphoma and chronic lymphocytic leukemia involving lipid rafts, Clin Canc Research. 2010; 16: 2046-2054.
11. Mollinedo F, Gajate C, Martín-Santamaría S, Gago F. ET-18-OCH3 (edelfosine): a selective antitumour lipid targeting apoptosis through intracellular activation of Fas/CD95 death receptor. Current medicinal chemistry.2004; 11: 3163-3184.
12. Fujiwara K, Koike H, Ohishi Y, Shirafuji H, Kohno I, Modest E, et al. Cytokinetic and morphologic differences in ovarian cancer cells treated with ET-18-OCH3 and the DNA-interacting agent, etoposide, Anticancer research. 1996; 17: 2159-2167.
13. Busto JV, del Canto-Jañez E, Goñi FM, Mollinedo F, Alonso A. Combination of the anti-tumour cell ether lipid edelfosine with sterols abolishes haemolytic side effects of the drug. J chem biol. 2008; 1: 89-94.
14. Rybczynska M, Spitaler M, Knebel NG, Boeck G, Grunicke H, Hofmann J. Effects of miltefosine on various biochemical parameters in a panel of tumor cell lines with different sensitivities. Biochem pharmacol. 2001; 62: 765-772.
15. Dummer R, Krasovec M, Röger J, Sindermann H, Burg G. Topical administration of hexadecylphosphocholine in patients with cutaneous lymphomas: results of a phase I/II study, J Am Acad Dermatol. 1993; 29: 963-970.
16. Khademvatan S, Gharavi MJ, Rahim F, Saki J. Miltefosine-induced apoptotic cell death on Leishmania major and L. tropica strains. Korean J Parasitol. 2011; 49: 17-23.
17. Ferreira AK, Meneguelo R, Pereira A, FILHO OMR, Chierice GO, Maria DA. Anticancer effects of synthetic phosphoethanolamine on Ehrlich ascites tumor: an experimental study, Anticancer Res. 2012; 32: 95-104.
18. Jiménez-López JM, Ríos-Marco P, Marco C, Segovia JL, Carrasco MP. Alterations in the homeostasis of phospholipids and cholesterol by antitumor alkylphospholipids, Lipids Health Dis. 2010: 9: 33.
19. Hąc-Wydro K, Dynarowicz-Łątka P. Searching for the role of membrane sphingolipids in selectivity of antitumor ether lipid–edelfosine, Colloids and Surfaces B: Biointerfaces. 2010; 81: 492-497.
20. Ngwenya BZ, Fiavey NP, Mogashoa MM. Anti-neoplastic action of peritoneal macrophages following oral administration of ether analogues of lysophospholipids. European J Cancer. 1992; 28: 1637-1642.
21. Sprong H, van der Sluijs P, van Meer G. How proteins move lipids and lipids move proteins, Nature Reviews Molecular Cell Biology. 2001; 2: 504-513.
22. van Blitterswijk WJ, Verheij M. Anticancer alkylphospholipids: mechanisms of action, cellular sensitivity and resistance, and clinical prospects, Current pharmaceutical design. 2008; 14: 2061-2074.
23. Gibellini F, Smith TK. The Kennedy pathway-de novo synthesis of phosphatidylethanolamine and phosphatidylcholine. IUBMB life. 2010; 62: 414-428.
24. Huang WC, Chen CL, Lin YS, Lin CF. Apoptotic sphingolipid ceramide in cancer therapy, J lipids. 2011.

Cite this article

Mambelli LI, Jorge SD, Pasqualoto K, Zaim MH, de Azevedo RA, et al. (2017) The New Age for Discovering New Selective Antitumor Agents: Antineoplastic Phospholipids. J Pharmacol Clin Toxicol 5(2):1072.