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The role of bioactive lipids in the development of soft tissue sarcomas in children – a review article

Rola bioaktywnych lipidów w rozwoju mięsaków u dzieci – przegląd piśmiennictwa

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SUMMARY
Soft tissue sarcoma accounts for almost 7% of all malignant tumours in children and adolescents. This heterogeneous group of mesenchymal and neuroectodermal tissue neoplasms can be characterized by common biological and clinics traits. Bioactive lipids are described as lipids, the levels of which – when changed – lead to changes in cell functioning. The role of bioactive lipids in the biology of neoplasmatic cells is unquestionable. In this paper we present an overview of articles describing the impact of selected bioactive lipids on the development of sarcoma in children.
Key words: bioactive lipids, soft tissue sarcoma, children.

STRESZCZENIE
Mięsaki tkanek miękkich stanową prawie 7% wszystkich złośliwych nowotworów rozpoznawanych u dzieci i młodzieży. Heterogeniczna grupa guzów rozwijająca się z tkanki mezenchymalnej i neuroektodermalnej charakteryzuje się zarówno podobnymi właściwościami biologicznymi, jak i cechami klinicznymi. Podkreśla się znaczenie lipidów bioaktywnych, ponieważ każda zmiana ich stężenia powoduje zmianę w funkcjonowaniu komórki. Niezmiernie istotny jest wpływ bioaktywnych lipidów na funkcjonowanie komórek nowotworowych.
W prezentowanej pracy przedstawiono rolę wybranych bioaktywnych lipidów w rozwoju mięsaków tkanek miękkich u dzieci na tle światowego piśmiennictwa.
Słowa kluczowe: lipidy bioaktywne, mięsaki, dzieci.

INTRODUCTION

Soft tissue sarcoma (STS) accounts for almost 7% of all malignant tumours in children and adolescents. This heterogeneous group of mesenchymal and neuroectodermal tissue neoplasms can be characterized by common biological and clinics traits [1]. Rhabdomyosarcoma is the most common STS in children. It arises from progenitor cells of skeletal muscles and makes up to 50% of all described STS. Other malignant changes of soft tissue in children can be divided into two groups: RMS-like tumours (synovial sarcoma, primitive neuroectodermal tumour, extra-costal Ewing sarcoma, undifferentiated sarcoma) and non-RMS tumours [2].

Due to similarities in STS symptoms, location and histology it is often necessary to conduct genetic tests, which are crucial to establish the diagnosis. Good examples are alveolar RMS (ARMS), where in 70% of cases translocations t (2:13; p36; q14) can be found, leading to the presence of PAX3-FKHR and PAX7-FHKR fusion genes [3].

Treatment strategies have been evolving for 30 years and allow for good effects when combining chemotherapy and radiotherapy with surgical procedures. The mean five-year survival rate for all STS is from 50% to 80% including 70% in RMS, 50% in peripheral primitive neuroectodermal tumour (PNET), and 80% in synovial sarcoma (SS). Upon establishing the diagnosis, distant metastases can be found in approximately 20% of patients. This finding decreases the effectiveness of treatment and makes for poorer prognosis. The mean five-year survival rate in those cases is 20–30%, but can be even lower for individuals with bone or bone marrow metastases [4].

Bioactive lipids are described as lipids, the levels of which – when changed – lead to changes in cell functioning. In the last two decades the most promising group of the abovementioned lipids comprise sphingolipids – present in every eukaryotic cell, and also in some prokaryotes [5]. The two substances of the large sphingolipid group discussed below are S1P and C1P. They are known to influence many cell functions, including those responsible for neoplasmatic growth. S1P stimulates cell proliferation and migration, leads to cytoskeletal actin rearrangement, and inhibits apoptosis. C1P increases inflammatory action by stimulating calcium-dependent mast-cell degranulation, and also increases cell survival by stimulating DNA synthesis [6, 7, 8]. Moreover, both of these bioactive lipids have been described as crucial to skeletal muscle development [9, 10].

This article aims to review the available literature dealing with the influence of bioactive lipids on the development and resistance to treatment of STS.

DISCUSSION AND REVIEW OF REFERENCES

A number of articles regarding the function of bioactive lipids in cell physiology during the development of various malignant tumours were reviewed. Special attention was paid to papers concerning STS in children.

Hokin and *Hokin* were the first to use the term "bioactive lipids" [11]. In the 1950s they observed and described a rapid turnover of inositol phospholipids in pancreatic slices that were stimulated with acetylcholine. Despite that fact, the role of diacerole (DAG) and inositol-1, 4, 5-triphosphate (Ins 1, 4, 5 P3) in the regulation of protein kinase C (PKC) and calcium releasing was discovered later. DAG–PCK activation was reported, and it established the opinion that lipids can regulate cellular signals [5].

Sphingolipids are conserved in all eukaryotic cells and some prokaryotic cells (e.g. in the bacterial genus Sphingomonas). Loss of sphingolipid biosynthesis causes cell death in yeast, and CHO cell embryonic lethality in fly and mouse [12]. They are built of an amide-linked fatty acid chain and an amino--alcohol sphingosine base. Sphingolipids are irregularly distributed in the plasmatic membrane. Laterally, sphingolipids and cholesterol form membrane lipid microdomains. Vertically, sphingolipids and glycerophospholipids are unequally distributed between two leaflets of the plasmatic membrane [13, 14].

Ceramides are the central elements of sphingolipids metabolism and they can be generated by two major mechanisms. One of them is the *de novo* pathways of sphingolipids metabolism, and has a unique metabolic entry point, serine palmitoyl transferase (SPT), which forms the first sphingolipid of the novo pathway and as a unique exit point of this process, S1P lyase, which breaks down S1P into non-sphingolipid molecules. The second mechanism is known as the "sphingomyelin cycle", and it is responsible for the production of ceramide, which involves hydrolysis of the plasma's membrane sphingomyelin to ceramide and phosphorylcholine throughout the action of sphingomyelinase [15, 16]. In mammals S1P is described as acting as a receptor ligand, an intracellular signalling molecule, and the intermediate form in sphingolipid-to-glycerophospholipid conversion [12].

Up to now, five S1P receptors have been identified: from S1P1 to S1P5. The S1P1 family receptors are G protein-coupled receptors (GPCRs) [17]. In mammals S1P1, S1P2 and S1P3 are expressed in all tissues, whereas S1P4 and S1P5 are only restricted to some. S1P4 is present in lymphoid tissue (thymus, spleen, lymph nodes), while S1P5 is present in the brain and skin. When S1P is binding to its receptor, it activates a downstream signalling pathway leading to a variety of cellular responses, such as proliferation, regulation of cell migration and actin cytoskeletal rearrangement, and adherents junction assembly [18, 19]. There is also a specific role of S1P and S1P1 in lymphocyte trafficking between the blood and lymphoid tissue [20, 21]. S1P is also known to be involved in the stimulation of chemotaxis cell survival and regulation of angiogenesis [22, 23].

C1P is reported to act as an intracellular signalling molecule. Up to now, no surface cell receptors for C1P have been described despite many researches suggesting that C1P plays an important role in the immune system, and is an exogenous factor for cell survival and DNA synthesis, and that it increases intracellular Ca^{2++} concentrations [5, 7, 24].

Soft tissue sarcomas usually occur in infants 2 to 6 years old and teenagers 15 to 19 years old. These tumours can develop in various areas of the body: head, neck, upper and lower extremities, pelvic area, genitourinary system, and in the chest and lungs. Certain locations can be characteristic for particular histological types. Sarcomas are considered to be locally malignant with their expanding growth and frequent recurrences in the primary location. These tumours are also able to cause distant metastases by haematogenous spread into the lungs, bone marrow and nervous system [1, 2, 25].

Sarcomas, especially rhabdomyosarcoma, are more frequent in children with inborn defects of the central nervous and genitourinary systems, gastrointestinal tract and cardiac defects, as well as in patients suffering from neurofibromatosis, and in Li-Fraumeni Syndrome-affected families [26, 27, 28]. Clinical symptoms depend on the location of the neoplasm but not on histological type, and hence there are no symptoms characteristic for any particular sarcoma. Most of the conditions are caused by either mechanical compression of other anatomical structures or expansion of the tumour [1, 2, 25]. The diagnosis should be established based on a detailed medical history, and physical and radiological examinations, as well as laboratory and molecular tests. The cancer staging notation is usually established based on the size and radicality of the resection, and the presence of distant metastases and the tumour's response to chemotherapy [1, 3, 29].

Treatment strategies for sarcomas were developed by the Intergroup Rhabdomyosarcoma Study (IRS) and the International Society of Paediatric Oncology (SIOP – MMT), Cooperative Weichteilsarkom – studiengruppe – Gesellschaft für Pädiatrische Onkologie und Hämatologie, (CWS – GPOH), and the Italian Cooperative Study Group – Associazione Italiana Ematologia Oncologia Pediatrica (ICG – AIEOP). The aim of the treatment is to achieve the best possible control over the local and systemic development of the disease. The therapy combines surgical treatment with chemotherapy and radiotherapy [30, 31, 32, 33]. So far, there is only one report regarding the influence of S1P and C1P on the cells of STS in children. In that study *Schneider et al.* described the influence of S1P and C1P on rhabdomyosarcoma cell lines, including both ARMS (RH4, RH5, 4H18, RH28, RH30, RH41, CW9019) and ERMS (JR, SMS-CTR, RD, RH36) [34].

The influence of bioactive lipids was evaluated regarding RMS cell proliferation, chemotaxis, phosphorylation of intracellular protein pathways, and adhesion to fibronectin and bone marrow derived stroma cells, as well as neoplasmatic growth in immunodeficient mice. The lack of S1P and C1P influence on RMS cell proliferation was without any doubt very surprising when considering earlier reports pointing to their stimulating role in skeletal muscle cell development. *Schneider et al.* highlighted the considerable influence of various bioactive lipids on cell migration. Further research has allowed

them to establish that the activation of S1P1 receptors on the surface of neoplastic cells is responsible for that fact. Moreover, inhibition of the S1P1 receptor in these cells leads to the similar effect of increased cell mobility [35]. Another curious piece of information regards the discovery of increased RMS cell adhesion to fibronectin influenced by both S1P and C1P. The increased adherence to BM-derived stroma cells was observed under the influence of S1P only.

The authors considered the prometastatic effect of radiotherapy and chemotherapy as one of side effects, and they measured levels of S1P and C1P in the cells of tissues frequently affected by RMS metastases (bone marrow, liver, brain, lungs) before and after the treatment of immunodeficient mice implanted with RMS cells and subsequently treated with radiotherapy and chemotherapy. A significant increase of S1P and C1P was observed in bone marrow exposed to radiotherapy/chemotherapy. All those observations may point to S1P and C1P as one of the RMS cell mobility factors and for its ability to cause distant metastases as an effect of chemotherapy and radiotherapy. Curiously, the same group of researchers conducted similar experiment with other bioactive lipids, LPA and LPC, observing their influence on RMS cells with the same results as presented above. This not only suggests the identification of one factor promoting distant metastases, but also new possibilities of supporting therapy in treating cancer. Various publications regarding different points of the sphingolipid metabolism that could be influenced by exogenous agents confirm that.

Another group of researchers focused on the applicability of the S1P1 receptor in the differentiation of neoplasms. It is known that this receptor can be found on the surface of endothelial cells. *Akiyama et al.* examined samples of various tumours in adults, 3 of which were samples of the STS group similar to the ones developing in children (angiosarcoma, liposarcoma, malignant fibrous histiocytoma). All tumours of a vascular background (haemangioma, lymphangioma, angiosarcoma) were positive for S1P1. However, sensitivity and specificity in angiosarcoma tissues were much higher in comparison to a commonly used marker – CD31. This means that the evaluation of the S1P1 receptor may be crucial for the correct differentiation of angiosarcoma with other tumours [36].

A different aspect of bioactive lipids' influence on neoplasmatic cells was researched by *Nofiele et al.* They revealed that in many of these cells – including mouse fibrosarcoma – ultrasound-stimulated microbubbles cause the increase of ceramides, which make these cells more sensitive to radiotherapy. This effect was nullified by exposing the abovementioned cells to physiological concentrations of S1P. Based on previous data, it can be concluded that the anti-apoptotic effect of S1P is directly linked with cell resistance to radiotherapy [37].

CONCLUSIONS

The role of bioactive lipids, including S1P and C1P, in the biology of neoplasmatic cells is unquestionable. All the above-mentioned publications show various possibilities for improving the diagnostics and therapy of STS, and enable a better understanding of the mechanism of bioactive lipids' activity in both healthy and neoplasmatic cells. It is without doubt an aspect offering new therapeutic options for children with STS.

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