Review paper

Betulin: a natural product with promising anticancer activity against colorectal cancer cells

Betulina – związek pochodzenia naturalnego o obiecującym działaniu przeciwnowotworowym w stosunku do raka jelita grubego

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Słowa kluczowe: rak jelita grubego, betulina, związki naturalne.

Abstract

Colorectal cancer is the third most frequently diagnosed type of cancer in men, while it ranks second in women. The high mortality rate of people with colorectal cancer is due to the advanced stage at diagnosis and the low selectivity of the currently used cytostatics. One of the important directions in searching for new anticancer substances is research of natural origin compounds. Betulin is a promising substance from this group, showing activity against colon cancer cells with low toxicity. Studies of betulin carried out in the aspect of the evaluation of this direction of activity concerned human tumor cell lines Col2, SW707, DLD-1, HT29 and HCT116. It was also important to determine the molecular mechanisms of action of this triterpene in colon cancer cells.

Streszczenie

Rak jelita grubego jest trzecim najczęściej diagnozowanym u mężczyzn rodzajem nowotworu, a drugim u kobiet. Wysoka śmiertelność osób z rakiem jelita grubego spowodowana jest zaawansowaniem w momencie zdiagnozowania oraz niską selektywnością obecnie stosowanych cytostatyków. Jednym z ważniejszych kierunków poszukiwania nowych substancji przeciwnowotworowych są badania związków pochodzenia naturalnego. Obiecującą substancją z tej grupy, cechującą się działaniem w stosunku do komórek raka jelita grubego przy jednocześnie niskiej toksyczności, jest betulina. Badania prowadzone pod kątem oceny tego kierunku aktywności dotyczyły ludzkich linii komórek nowotworowych Col2, SW707, DLD-1, HT29 i HCT116. Istotne było również określenie molekularnych mechanizmów działania tego triterpenu w komórkach raka jelita grubego.

Introduction

Colorectal cancer (CRC) is diagnosed in approximately one million people each year worldwide. It is the third most common cancer in men and the second in women. Moreover, it is the second leading cause of death in Europe [1]. Over the last 30 years a significantly growing incidence of CRC has been observed in Poland [2]. According to the statistics of the National Cancer Registry, in 2017, 10,905 cases of illnesses and 7,754 deaths due to colon cancer were recorded in Poland [3]. With age, the number of diagnosed cases of this neoplasm gradually increases. About 90% of CRC cases are due to an accumulation of mutations or epigenetic modifications in certain genes. The others are so-called familial CRC cases, namely Lynch syndrome (LS) and familial adenomatous polyposis (FAP).

The development of this neoplasm results from changes at the genetic and epigenetic level as well as the occurrence of certain non-genetic factors affecting the disturbance of homeostasis in the large intestine. This leads to the disruption of intracellular signaling pathways and the loss of cell control over processes such as proliferation and apoptosis. The main risk fac-

tors for colorectal cancer are excessive consumption of alcohol and red meat, a diet low in fiber, obesity and smoking. A lack of physical activity also contributes to the development of this cancer [1–4]. The basic method used in the treatment of colorectal cancer is surgical resection of the tumor, which can be performed using a classic or laparoscopic method. Despite many screening programs to reduce the incidence of CRC, many cases are diagnosed with advanced metastatic disease [5]. Complementary to the treatment of advanced colorectal cancer is chemotherapy or radiochemotherapy. Unfortunately, chemotherapy is associated with limitations of various kinds, such as systemic toxicity or tumor resistance. In the treatment of colorectal cancer targeted drugs (5-fluorouracil, irinotecan, oxaliplatin, capecitabine, panitumumab, cetuximab, bevacizumab) are used in combination with other substances or individually [6]. The high mortality rate in CRC is largely due to low selectivity of currently used anticancer drugs [7]. The implementation of new methods of treatment and the search for new potential anticancer substances, including from the group of compounds of natural origin, aim to improve the effectiveness of treatment.

Natural products with cytotoxic effects on colon cancer cells

Natural anticancer compounds have been used in traditional medicine since ancient times. These substances are isolated from different kinds of biological material (plants, fungi, marine animals or microorganisms) and are characterized by a diverse chemical structure. The compounds of natural origin have the ability to model signaling pathways and regulate gene expression related to the cell cycle. Therefore, they can influence cell differentiation and apoptosis. These compounds can be used in the treatment of neoplastic diseases in combination with the chemotherapeutic agent in the so-called combination therapy. Replacing a part of the dose of a classic anticancer drug with a natural substance, often with a different mechanism of action, may reduce the toxic burden on the patient's body and drug resistance [8, 9].

The conducted studies indicate that many plant-derived compounds have potential in the treatment of CRC. This group of natural products includes among others berberine, butein, capsaicin, cardamonin, celastrol, diosgenin, escin, curcumin, silibinin, betulin and betulinic acid (Figure 1) [10, 11].

Betulin

Promising anticancer compounds are the pentacyclic triterpenes of the lupane type, a large group of secondary metabolites derived from plants. A representative of this class of compounds is betulin isolated from the outer bark of the birch through sublimation by Lowitz in 1788. Betulin is a lipophilic compound which results from the presence of four six-membered rings and one five-membered ring in its structure. Betulin has three reactive groups at positions C-28 (primary hydroxyl group), C-3 (secondary hydroxyl group), and the isopropenyl moiety at position C-19, which makes it possible to modify its chemical structure. It is worth noting that betulin, like its oxidized form (betulinic acid) has low toxicity. Unfortunately, low solubility in water limits the application of this triterpene in medicine [12, 13]. Betulin is characterized by a broad spectrum of biological activity including antitumor, antiviral and antibacterial action. Nevertheless, the most promising activity of this compound is its antitumor potential against various types of human cancers, including those related to the digestive system, such as colorectal cancer [14].

The anticancer effect of betulin against human colorectal adenocarcinomas depends largely on the type of cell line (Table 1). Betulin isolated from the species *Celtis philippensis* widely distributed in Africa, Asia and Australia was tested on human colon cancer cells Col2. This compound was found to be less cytotoxic (ED_{50} > 45 μM) against the Col2 cell line compared to taxol and camptothecin applied as reference substances [15]. Similarly, for betulin obtained from *Betula verrucosa*, low cytotoxic activity was observed in colorectal adenocarcinoma SW707 cells with an IC_{50} value of 51.7 μM [16]. A much better result was obtained in the study of betulin isolated from the outer bark of *Betula papyrifera* in relation to the human colorectal adenocarcinoma cell line DLD-1. The IC_{50} value determined for betulin was 6.6 μM and was almost two times better than for betulinic acid, which was used as a reference in this study [17]. The cytotoxic activity of betulin towards the human colorectal adenocarcinoma HT29 cell line was also confirmed. Studies of the antitumor activity of betulin in HT29 cells showed significant differences in IC_{50} values ranging from 4.3 μM up to < 30 μM [18–20]. Recent studies are related to the determination anticancer properties of betulin against human colon cancer cells and the explanation of its mechanisms of action at the molecular level (Table 2). It was found that betulin significantly reduces the viability of HCT116 and HT29 cells at concentrations of 11.3 μM and 22.6 μM. Increased levels of cleaved caspases -9, -3 and -8 in HCT116 and HT29 cells treated with betulin were observed. Betulin triggers apoptosis in HT29 and HCT116 cells by activating the caspase-3 and -9 pathways. Moreover, release of cytochrome c was detected in the cytosolic area of cells [21].

An important aspect of the research was to determine the antitumor activity of betulin against metastatic CRC cells. *In vivo* studies have shown that betulin inhibits lung metastasis by cell cycle arrest, inducing autophagy and apoptosis of metastatic colorectal can-

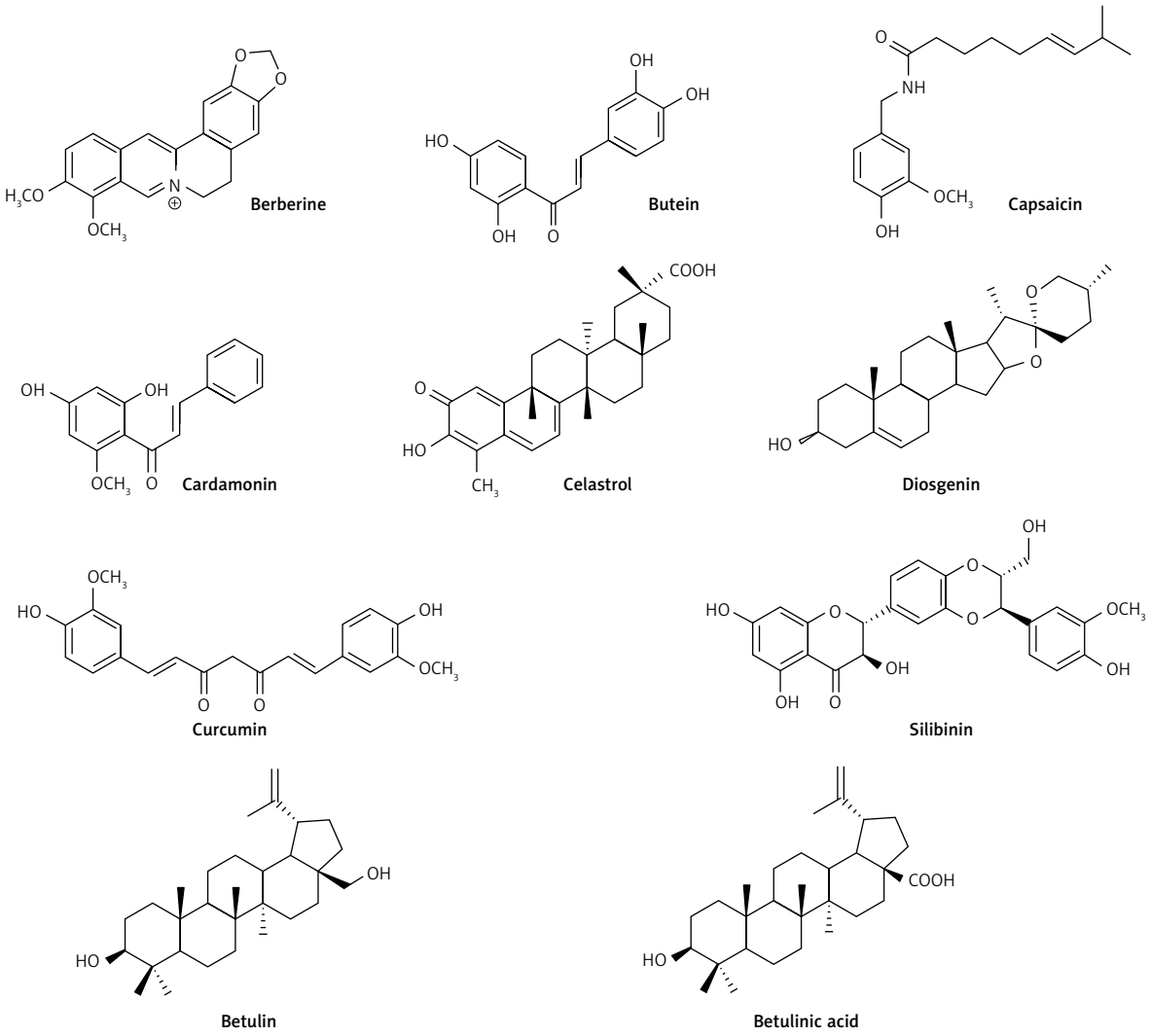

Figure 1. Chemical structures of plant-derived compounds with activity towards colorectal cancer

cer cells. Introducing betulin into metastatic CRC cells causes arrest of their cell cycle in the G0/G1 phase by activating AMPK. Further studies showed that autophagy via AMPK and PI3K/Akt/mTOR signaling is one of the possible mechanisms of betulin antiproliferative activity in HCT116 cells. Identification of the molecular mechanisms involved in betulin-induced apoptosis in HCT116 cells was determined by analysis of MAPK phosphorylation. It has been demonstrated that betulin can suppress ERK, JNK, and p38 phosphorylation in HCT116 cells. The apoptotic effect of betulin on colon cancer cells may be mediated by the MAPK signaling pathway [22].

Betulin is a compound widely distributed in the natural environment. Scientific interest in this pentacyclic triterpene results from the multidirectional biological activities, mainly from its anticancer activity. Despite the promising pharmacological profile, the application of betulin in clinical use is limited. To improve the bioavailability of betulin, which correlates with its low water solubility, several nanoformulations have been developed. Application of nano-

Table 1. Anticancer activity of betulin towards various colorectal cancer cells

Cell line	IC_{50} or ED_{50} [μM]	Reference
Col2	45*	[15]
SW707	51.7	[16]
DLD-1	6.6	[17]
HT29	4.3	[18]
HT29	< 20	[19]
HT29	< 30	[20]

**ED_{50} value.*

Table 2. Molecular mechanism of action of betulin towards various colorectal cancer cells

Molecular mechanism	Cell line	Reference
Induces apoptosis, activating the caspase-3 and -9 pathways	HT29	[21]
Induces apoptosis, activating the caspase-3 and -9 pathways	HCT116	[21]
Induces cell cycle arrest Induces autophagy via AMPK and PI3K/Akt/mTOR signaling Induces apoptosis by decreasing phosphorylation of MAPKs	HCT116**	[22]

***Metastatic CRC.*

formulations with betulin (cyclodextrin complexes, nanoemulsions, nanostructured carbon sorbent, gold nanoparticles, polymer-liposome nano-complexes) improved the pharmacokinetic profile of this compound. Thanks to this, it is possible in the future to use betulin as a compound of low toxicity in the treatment of diseases related to the neoplastic process [23].

Summary

The increase in the incidence of neoplastic diseases in modern society necessitates the search for more effective and less toxic medicinal substances. The high incidence and mortality rate of colorectal cancer are particularly worrying. Alternatives to currently used anticancer drugs are naturally occurring compounds with activity against CRC. An interesting object of research in the group of compounds of natural origin showing activity against CRC is betulin, which belongs to the pentacyclic triterpenes of the lupane type. The antitumor activity of this triterpene against Col2, SW707, DLD-1, HT29 and HCT116 colon cancer cells was confirmed. The differences in betulin activity against colorectal cancer are due to the type of cell line. Significant betulin activity against DLD-1 and HT29 cells was observed. An important element of the research is to determine the molecular mechanisms of antitumor activity of betulin in colon cancer cells. Betulin in HCT116 cells induces apoptosis by activating the caspase-3 and -9 pathways or decreasing phosphorylation of MAPKs. Additionally, in HCT116 cells betulin may induce autophagy via AMPK and PI3K/Akt/mTOR signaling.

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Conflict of interest

The authors declare no conflict of interest.

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