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Metformin – a new approach

Metformina – nowe podejście

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Abstract

Metformin is a widely used biguanide drug recommended as a first-line antidiabetic for type 2 diabetes. Currently, metformin is used not only in the treatment of diabetes but also in other diseases. Some studies have shown that metformin causes weight loss in insulin-sensitive and insulin-resistant overweight and obese patients. Metformin is an effective and safe option for women with gestational diabetes and type 2 diabetes in pregnancy, and it may also increase the ovulation rate in patients with polycystic ovary syndrome (PCOS). Longer survival times have been observed in cancer patients using metformin. Metformin has been shown to significantly correlate with lower mortality in obese or type 2 diabetic women hospitalized for COVID-19. It also has a protective effect on the development and progression of many types of cancer. The mechanisms of action of metformin are complex and still not fully understood. Metformin has been shown to act through both AMP-activated protein kinase (AMPK)-dependent mechanisms and AMPK-independent mechanisms. This paper presents the benefits of using metformin in the treatment of various diseases.

Key words:

metformin, cancer disease, obesity, PCOS, vitamin B_{12}.

Streszczenie

Metformina jest szeroko stosowanym lekiem biguanidowym, zalecana jako terapia farmakologiczna pierwszego rzutu u pacjentów z rozpoznaniem cukrzycy typu 2. Obecnie metformina stosowana jest nie tylko w leczeniu cukrzycy, lecz także w przypadku innych schorzeń. Niektóre badania wykazały, że metformina powoduje zmniejszenie masy ciała u pacjentów z nadwagą i otyłością, którzy są wrażliwi i oporni na insulinę. Metformina jest skuteczną oraz bezpieczną opcją terapeutyczną dla kobiet z cukrzycą ciążową i cukrzycą typu 2 w ciąży, może także zwiększać wskaźnik owulacji u pacjentek z zespołem policystycznych jajników (PCOS). U pacjentów przyjmujących metforminę zaobserwowano dłuższy czas przeżycia. Wykazano także, że metformina istotnie koreluje z mniejszą śmiertelnością u kobiet z otyłością lub cukrzycą typu 2 hospitalizowanych z powodu COVID-19. Ma ona również ochronny wpływ na rozwój i progresję wielu typów nowotworów. Mechanizmy działania metforminy są złożone i wciąż nie do końca poznane. Wykazano, że metformina działa zarówno poprzez mechanizmy zależne od kinazy białkowej aktywowanej przez AMP (AMPK), jak również przez mechanizmy, które są niezależne od AMPK. W pracy przedstawiono korzyści płynące z zastosowania metforminy w leczeniu różnych chorób.

Słowa kluczowe:

metformina, choroby nowotworowe, otyłość, PCOS, witamina B_{12}.

Introduction

Metformin (N, N-dimethyl biguanide) belongs to the biguanide class of anti-diabetic drugs. These drugs contain 2 linked guanidine rings. The main target tissue of metformin is the liver, and its main effect is the reduction of glucose secretion in the liver due to the inhibition of gluconeogenesis, which leads to a reduction in blood glucose levels [1]. Metformin slows intestinal glucose absorption [2]. In addition, metformin indirectly increases insulin sensitivity by increasing peripheral glucose utilization [1]. Metformin rarely causes side effects such as hypoglycaemia, hyperinsulinaemia, vitamin B_{12} deficiency, peripheral neuropathy, or acidosis, comparing to other antidiabetic drugs [3]. Other common side effects of metformin include loss of appetite, epigastric pain, nausea, and diarrhoea [4]. The US Food and Drug Administration (FDA) approved metformin in 1994 for the treatment of type 2 diabetes [3]. Currently, metformin is recommended as the first-line treatment for type 2 diabetes [5]. Metformin is mainly absorbed from the small intestine but is excreted unchanged in the urine. The elimination half-life of metformin during multiple dosages in patients with good renal function is approximately 5 hours [6].

Biodistribution studies of metformin in human target tissues were performed using labelled metformin – 11C. Positron emission tomography (PET) showed that ^{11}C-metformin was primarily taken up by the kidneys, urinary bladder, and liver, but also to

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a lesser extent by salivary glands, skeletal muscles, and intestines. Hepatic uptake of ^{11}C-metformin was pronounced after oral administration of the tracer withet al.tissue-to-blood ratio double that which was noticed after intravenous administration. However, only a slow accumulation of ^{11}C-metformin was found in muscles [7].

The mechanisms of action of metformin are complex and still not fully understood. Metformin reduces liver glucose production, but many studies suggest that metformin also plays a key role in the gut. Metformin has been shown to act via both AMP-activated protein kinase (AMPK)-dependent mechanisms and AMPK-independent mechanisms – by inhibition of mitochondrial respiration, but also possibly by inhibition of mitochondrial glycerophosphate dehydrogenase, and mechanisms involving lysosome [2].

Recently, it was thought that metformin improves glycaemia by acting on the liver via AMPK activation, but it is now known that the action of metformin is more complex and involves different modes of action [2].

Overweight and obesity

Metformin is an effective drug to reduce body weight in insulin-sensitive and insulin-resistant overweight and obese patients. The mean weight loss in the metformin-treated group was higher compared to the untreated control group. The percentage of weight loss was independent of age, BMI, or sex [8]. In 2021, the American Diabetes Association recommended that metformin therapy for the prevention of type 2 diabetes should be considered in people with pre-diabetes, especially for those with a BMI $\geq$ 35 kg/m^2, those aged $<$ 60 years, and women with prior gestational diabetes mellitus [9].

Gestational diabetes

Many studies have shown that metformin is an effective, safe, and low-cost option for women with gestational diabetes and type 2 diabetes in pregnancy. The obtained results suggest that metformin can improve maternal and neonatal outcomes [10–13]. In the group of patients treated with metformin during pregnancy, a lower increase in maternal weight was observed ($p < 0.001$), as well as a lower frequency of neonatal hypoglycaemia and hospitalization of newborns in the intensive care unit [11]. Rowan *et al.* compared the body composition and metabolic outcomes at 7–9 years in the offspring of women with gestational diabetes (GDM) treated with metformin or with insulin during pregnancy. Analysis showed similar percentages of total and abdominal fat and similar metabolic parameters in offspring in both groups. However, children of mothers taking metformin during pregnancy at the age of 9 years had higher values of body weight, arm and waist circumference, and waist circumference-to-height ratio [12]. Moreover, the use of metformin during pregnancy in women with polycystic ovary syndrome reduces the incidence of early pregnancy loss and preterm labour, and protects against foetal growth restriction [10].

Polycystic ovary syndrome

The results of many studies show that metformin increases the ovulation rate in patients with polycystic ovary syndrome (PCOS) as compared to placebo. In 2017, the American Society for Reproductive Medicine published guidelines that did not recommend metformin as first-line therapy for anovulation, because oral ovulation-inducing drugs such as clomiphene citrate or letrozole are much more effective at increasing ovulation, pregnancy, and the birth rate in women with PCOS. However, it has since been confirmed that metformin alone does not increase the rate of miscarriage when its use is discontinued early in pregnancy. Nonetheless there is insufficient evidence that metformin in combination with other agents that induce ovulation increases live birth rates [14].

Survival time and mortality

Metformin affects lifespan. A meta-analysis involving over 800,000 patients found that metformin use was associated with lower rate of all-cause mortality [15]. Bannister *et al.* compared the mortality in a group of patients with diabetes who use metformin or sulphonylurea monotherapy with that in a control group of persons without diabetes. Diabetic patients using metformin alone lived longer. Median survival time in the nondiabetic group was 15% shorter, while in the sulphonylurea monotherapy group it was 38% shorter compared to the metformin group. These results support the use of metformin as the first-line therapy and suggest that metformin may also be beneficial in the non-diabetes population [16]. Another study found that diabetic patients using metformin had significantly lower all-cause mortality compared to non-diabetic patients. In addition, people using metformin compared with non-diabetes have a lower incidence of cancer (rate ratio = 0.94, 95% CI: 0.92–0.97). The reduction in mortality was associated with the use of metformin, which suggests that metformin may have a protective effect in the elderly, improving their health and prolonging their life [17].

In 2020 a meta-analysis evaluating the effect of metformin on the survival of women with ovarian cancer was published. The survival rate of female patients using metformin was compared with the survival rate of patients not using metformin. Six studies showed an association between the use of metformin and better survival rates, but all cases were burdened with immortal time bias (ITB). Only 2 studies were classified as immortal time bias-free (ITB-free). The overall survival benefit in metformin users was not observed in these studies was not observed overall survival benefit in metformin users. However, there was significant heterogeneity between the studies, possibly due to the difference in the exposure time to metformin [18].

COVID-19

Type 2 diabetes and obesity are chronic inflammatory states that are risk factors for the severe course of COVID-19 disease. Metformin has sex-specific immunomodulatory and cytokine-

reducing effects. In a retrospective cohort analysis, it was assessed whether metformin use reduced mortality in hospitalized COVID-19 patients. Patients included in the analysis were aged 18 years or older, had type 2 diabetes or obesity, and were admitted to the hospital for COVID-19, confirmed by PCR. Home use of metformin for more than 90 days during the year before hospital admission was an independent variable in the analysis. Metformin use was not associated with a significant reduction in mortality in the overall male and female population studied according to the stratified Cox proportional hazards model. However, in obese or type 2 diabetic women admitted to hospital because of COVID-19, metformin was associated with significantly lower mortality. The authors suggest that more prospective studies are needed to elucidate the mechanisms and causality. If further studies are repeatable, metformin could be widely used to prevent COVID-19 mortality because it is safe and inexpensive [19].

Metformin and vitamin B_{12} levels

Many studies have shown that metformin reduces the level of vitamin B_{12} [20–24]. In 2016, a systematic review and a meta-analysis were performed, which assessed the relationship between the use of metformin and vitamin B_{12} deficiency in people with type 2 diabetes. These studies included patients of any age or sex, taking any dose of metformin for any period. Ten observational studies showed statistically significant lower levels of vitamin B_{12} in patients treated with metformin compared to patients not on this drug [23]. In a study by Gupta *et al*., 50 patients with type 2 diabetes treated with metformin for at least 3 months had a significant negative correlation between the duration of metformin use and vitamin B_{12} levels ($r = -0.40$) [25]. There was also a positive correlation between the duration of metformin treatment and peripheral neuropathy ($r = 0.40$). A pilot randomized controlled trial conducted in 2019, assessing the effect of metformin on the level of vitamin B_{12} in 165 women receiving metformin or placebo for 3 years, showed a significant overall reduction in total serum vitamin B_{12} levels after metformin treatment. One quarter of patients using metformin and 1/8 of patients using placebo had a reduced level of vitamin B_{12} [26]. Thus, patients using metformin are at high risk of vitamin B_{12} deficiency, and so monitoring of vitamin B_{12} levels and screening for neuropathy is recommended.

Metformin and cancer

A meta-analysis published in 2018, including 20 million people, found that diabetes is a risk factor for cancer of any location in both men and women [27]. The mechanisms linking diabetes to cancer may include hyperglycaemia and hyperinsulinaemia (endogenous or exogenous) as well as insulin-like growth factor changes, chronic subclinical inflammation, disorders of sex hormone metabolism, adipokines, and possibly the effect of anti-diabetic drugs used to treat type 2 diabetes [28]. The results of many studies indicate that metformin has a protective effect on the development and progression of cancer [29–33]. Experimental studies performed on cancer cell lines, including monocytic human leukaemia cell line (THP-1), human acute lymphoblastic leukaemia acute line (CCRF/CEM), human lung adenocarcinoma cell line (A549), breast cancer cell line (MCF-7), and breast cancer doxorubicin-resistant cell line (MCF-7/DX), showed a positive effect of metformin in reducing cell proliferation. These results require further studies, but the results are promising [34]. It has been reported that the use of metformin is associated with a reduction in the risk of cancer: colorectal cancer [35–40], breast cancer [41, 42], lung cancer [43], liver cancer [44], stomach cancer [45, 46], and skin cancer [47]. A 2019 meta-analysis compared the cancer risk in patients with type 2 diabetes using metformin monotherapy compared to patients using sulphonylurea monotherapy. A total of 8 cohort studies were included in the meta-analysis. It was shown that metformin monotherapy in patients with type 2 diabetes was associated with a lower risk of cancer incidence compared to sulphonylurea monotherapy [48]. Metformin was found to improve the tumour cell response to radiation therapy in patients with various types of cancer and diabetes. Moreover, in these studies it was shown that the 2-year and 5-year survival of patients using metformin is longer compared to non-users of metformin. However, the authors suggest that the published results should be interpreted with caution because retrospective data are at risk of bias [49]. Dankner *et al*. conducted a study of 320,000 diabetic patients aged 21–87 years to assess the association between metformin and cancer incidence. The results obtained from the analysis did not confirm the association between metformin treatment and incidence of major cancers (excluding prostate and pancreas) in diabetic patients [50]. The results assessing the effect of metformin on cancer are inconclusive; therefore, it seems reasonable to conduct further randomized, long-term studies.

Breast cancer

Breast cancer now is most common form of cancer worldwide. According to a 2020 report, the incidence of breast cancer comprised 11.7% of all types of cancer [51]. It was shown that metformin is effective in inhibiting the proliferation and metastasis of breast cancer cells and inducing their apoptosis following multiple pathways [52, 53]. One of the mechanisms of metformin's anti-tumour action is the activation of adenosine monophosphate-activated protein kinase (AMPK), which is also involved in the anti-diabetic effect of metformin. It has been suggested that activation of AMPK leads to inhibition of the mammalian target of rapamycin (mTOR) and downstream pS6K, which regulates cell proliferation. Due to its hydrophilic and cationic nature, metformin requires cation-selective transporters to enter cells and activate AMPK [52]. Although AMPK activation is a key target for metformin's anti-tumour activity, there is evidence that metformin is also independent of AMPK. Metformin has been shown to inhibit the PI3K/Akt/mTOR signalling pathway. Moreover, metformin may exert its anti-tumour effects also through modulation of miRNAs, small non-coding RNAs approximately 20-25 nucleotides in length [53]. A meta-analysis based on the results of studies of over 838,000 participants,

published in 2015, showed that metformin treatment was associated with a reduction in all-cause mortality (RR, 0.652; 95% CI: 0.488-0.873; $p = 0.004$) [41]. It was also found that the use of metformin in the test group, compared to the control group, was not associated with a reduced incidence of breast cancer (RR 0.964; 95% CI: 0.761–1.221; $p = 0.761$) [41]. *In vitro* studies performed in human doxorubicin resistant breast cancer cell line (MCF-7 DX) have shown that metformin administration can reverse multidrug resistance (MDR) by reducing P-gp activity. The results of this study suggest that metformin can be used as an adjuvant in breast cancer chemotherapy [54]. In 2019, the impact of type II diabetes and certain diabetes on the risk of different molecular subtypes of breast cancer was assessed in a retrospective, multicentre study of over 4500 women with breast cancer [55]. Long-term use of metformin (13–24 months of treatment in the 24 months prior to diagnosis of breast cancer) has been shown to be associated with a 38% increased probability of developing triple-negative breast cancer (TNBC) [55]. The authors of these studies suggest that diabetes may be more strongly associated with the risk of triple-negative disease.

Lung cancer

Lung cancer is the second most common cancer in the world. According to the 2020 report, the incidence of lung cancer was 11.4% of all types of cancer [51]. It has been shown that metformin is also important in the prevention of lung cancer and in improving the survival of patients with lung cancer [56–59]. Many *in vitro* and *in vivo* studies indicate that metformin has a strong antitumour effect. Metformin in combined with another drug increases the effectiveness of chemotherapeutic agents and radiotherapy. The concentration of metformin used in *in vitro* studies was usually 1–10 mM, and in some studies it was even 50 mM [60]. Another study investigated whether the use of metformin in patients with non-small cell lung cancer and type 2 diabetes was associated with an improvement in patients' survival. The mean follow-up was 14.6 months. It was shown that the use of metformin after diagnosis (1 year of use) resulted in a significant reduction in mortality. Moreover, the reduction in mortality was especially pronounced only among patients who were also using metformin before lung cancer diagnosis and among patients at an early stage of diagnosis. The results of this study indicate that prolonged use of metformin in the study population was associated with improved survival, especially among patients in the early stages of the neoplastic disease [61].

Colon cancer

Colorectal cancer is the third most common cancer in the world. Data from a 2020 report show that the incidence of colorectal cancer comprised 10% of all types of cancer [51]. Metformin increases intestinal glucose uptake and lactate production. Administration of metformin increases GLP-1 concentration and the bile acid pool in the intestine, and alters the microbiome [62]. These changes may contribute to the inhibition of the development of colorectal cancer. The results of many studies indicate that metformin may play an important role in reducing the incidence of colorectal cancer [36–40]. The analysis of the Markov model showed that patients with type 2 diabetes treated with metformin had a lower incidence of colorectal cancer compared to patients not using metformin (1.670% vs. 2.146%, respectively; $p = 0.016$) [38]. Du *et al.* conducted a meta-analysis and showed that taking metformin is associated with improvement in overall survival (OS) and cancer-related time (CS) survival in diabetic colorectal cancer patients. A significant benefit for overall survival was observed in patients with stage II and III disease [63]. In 2020, Ng *et al.* analysed the results of 58 studies evaluating the effect of metformin on the incidence of colorectal adenoma and cancer; they found that people using metformin had a significantly lower incidence of colorectal adenoma (RR 0.77, CI: 0.67–0.88, $p < 0.001$), advanced adenoma (0.61, CI: 0.42–0.88, $p = 0.008$), and colorectal cancer (CRC) (RR 0.76, CI: 0.69–0.84, $p < 0.001$) compared with non-metformin patients. Overall survival (HR 0.6, CI: 0.53–0.67, $p < 0.001$) and CRC-specific survival (HR 0.66, CI: 0.59–0.74, $p < 0.001$) were longer among patients using metformin compared with non-metformin users. The overall survival analysis of patients with metastatic CRC revealed significantly higher survival rates in metformin users (HR 0.77, CI: 0.68–0.87, $p < 0.001$). It was confirmed that the use of metformin significantly reduces the incidence of colorectal adenoma and cancer, and improves the results of colorectal cancer treatment [39].

Synergistic action of metformin

It has been shown that the use of metformin in combination with other anti-cancer drugs can potentiate their effects. This is important because metformin is well tolerated and can practically lower the doses of cytotoxic chemotherapeutic agents in combination therapies without losing efficacy [3]. The combination of metformin with flavone caused a significant inhibition of cell viability and increased apoptosis of human breast cancer cells compared to metformin or flavone alone [64]. In another study in a mouse model, animals with transplanted breast cancer tumours received in their drinking water i.a. hemin and metformin [65]. Combination therapy of hemin and metformin has been shown to significantly inhibit tumour growth through the degradation of BACH1 and may be helpful in the treatment of triple-negative breast cancer (TNBC) [65]. Other studies have shown that metformin in combination with quercetin synergistically inhibits the growth, migration, and invasion of PC-3 and LNCaP prostate cancer cells. Combined use of quercetin with metformin induced more apoptosis than did single-agent treatment [66]. Metformin in combination with aspirin significantly inhibited the growth of pancreatic cancer cells in both *in vitro* and *in vivo* studies. In *in vitro* studies, the combination of metformin and aspirin significantly inhibited cell migration and pancreatic cancer cell colony formation – PANC-1 and BxPC3 – compared to untreated cells or compared to cells treated with single compounds. Moreover, *in vitro*, metformin and aspirin showed synergistic inhibitory effects on cell viability and induction of apoptosis. On the other hand, in *in vivo* studies, the com-

bination of metformin and aspirin significantly inhibited tumour growth and decreased the expression of Mcl-1 and Bcl-2 proteins in tumours [67]. In 2021, Anselmino investigated the effect of the combination of metformin and propranolol in the treatment of colorectal cancer and triple-negative breast cancer. *In vitro* studies showed that the combination of metformin and propranolol not only had an antiproliferative effect on colorectal cancer cells, but also decreased the ability of cells to form colonies and promoted apoptosis. Similar results were obtained in *in vivo* studies: it was observed that the combination treatment resulted in a significant reduction in the growth of CT116 and CT26 colon tumours in mice, without signs of toxicity. Moreover, the authors found that the combination of metformin and propranolol reduced the metastasis and proliferation of 5-FU-resistant colon cells [68]. In other studies of these authors it was found that the combination of metformin and propranolol was effective in preventing the growth of triple-negative breast cancer (TNBC) and the development of metastasis [69]. Ulrike Wokoun *et al.* investigated whether the combination of metformin and 2-deoxy-D-glucose increased antitumor efficacy in *in vitro* studies. Findings indicate that the combined use of metformin with 2-deoxy-D-glucose resulted in a significant reduction compared to single drugs. Moreover, a stronger induction of apoptosis was observed in the case of combination therapy compared to the treatment with a single drug [70].

Summary

Metformin is currently approved for the treatment of type 2 diabetes but has recently been reported to have therapeutic potential in the treatment of other diseases. The anti-aging and anti-tumour effects of metformin seem to be promising. Metformin causes weight loss in overweight and obese patients, and also positively affects outcomes in pregnant women. In addition, significantly lower all-cause mortality has been observed in patients using metformin, including obese or diabetic women hospitalized for COVID-19.

It should also be indicated that patients taking metformin are at an increased risk of vitamin B_{12} deficiency, so monitoring of vitamin B_{12} levels is important. Metformin in combination with other drugs may enhance their anti-cancer effects. This is important because metformin is well tolerated and can effectively reduce the dose of cytotoxic chemotherapeutic agents in combination therapies without reducing their anti-cancer efficacy. Metformin appears to have a protective effect on the development and progression of cancer, although the results of other studies do not confirm this relationship. Due to the inconclusive results, it seems advisable to conduct further, long-term randomized trials to confirm the effectiveness of metformin in the treatment of various diseases, as well as explain the various mechanisms of metformin action.

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