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Metformin — today's *panacea?*

ABSTRACT

Metformin is still an immutable basic hypoglycemic drug used in pharmacotherapy of diabetes mellitus type 2. Structurally it belongs to biguanide derivatives that was discovered in traditional herbalism. Galega officinalis was a plant used to obtain substances similar to metformin. This drug can induce a state in a cell, which occurs as a result of caloric restriction. It consists in decreasing the effectiveness of IGF-1 metabolic pathway with the insulin-like growth factor 1 and mTOR kinase activity, thus metformin contributes to extend a lifespan among animals. This article presents a brief overview of studies about anti-aging potential of metformin. Beside this, the authors have described caloric restriction role taking part in a lifespan as well as slowing down incidence of age related diseases. (Clin Diabetol 2016; 5, 4: 117–122)

Key words: metformin, aging, mTOR kinase, IGF-1 pathway, studies

Introduction

The number of elderly people steadily increases in recent decades. Primarily, it is a result of a significant development of medical science that we have been and still are witnessing. We cannot eliminate the aging process, however its retardation and thus slowing down the progress of age-related diseases is to improve the quality of life and its extension in good health. We cannot forget also about the economic dimension. Therefore, there is an upward trend in the number of scientific studies concerning the aging process, interference in the regulation of these processes and their most accurate recognition [1]. The scientists are also trying to develop new drugs or, what is significant, to find anti-aging potential among the drugs widely used in medical treatment of other diseases. One of them is metformin — a drug having pleiotropic effects in the body. The authors of this paper decided to focus on its activity.

Metformin is one of the most popular medicinal substances used in pharmacotherapy of diabetes mellitus type 2. Metformin monotherapy constitutes the first stage of treatment of this disease (together with the prescription to modify the lifestyle and reduce the calorific value of meals). Moreover, the place of metformin at a later stage of therapeutic process among other drugs (oral antidiabetics or insulin) is still well established [2]. The maximum therapeutic dose of metformin is 3000 mg/d and it is administered mostly in 3 divided doses. Its discovery dates back to Middle Ages, when the use of *Galega officinalis* in the traditional herbal medicine was associated with the treatment of symptoms resulting from diabetes mellitus type 2 [3]. Nevertheless, the clinical antidiabetic effect of metformin was discovered and proved in the 60's of the twentieth century, in the research carried out under the guidance of a French physician — Jean'a Sterne'a [4]. However, the clinical indications for the use of biguanide derivative do not limit only to the treatment of diabetes mellitus type 2. We should also mention the pre-diabetes condition, metabolic syndrome (or its components, including insulin resistance), polycystic ovary syndrome and lipodystrophy [5].

Among the current scientific studies concerning other uses of metformin, its anti-tumour and anti-aging activity is still mostly subject to evaluation. A growing number of reports in this regard proves that both the scientists and clinical practitioners are more and more

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interested in this issue. The fact is that, metformin has proven to be a drug which, during its use by patients with diabetes mellitus type 2, is also associated with a reduced risk of cancer and cardiovascular disorders. The efficacy of metformin in these diseases shows that it is possible to use a biguanide derivative in other diseases, and what is more, its anti-aging activity cannot be excluded *per se* [6].

Caloric restriction

Moderate caloric restriction is considered as the major modifiable factor which most significantly contributes to the prolongation of life in good health, reduces the incidence of diseases associated with age and it determines a better quality of life [7, 8]. The biochemical pathway with the somatotropic axis, i.e. the growth hormone, GH and insulin-like growth Factor 1, IGF-1 is one of the main biochemical pathways that actuate in response to a specific "sensor" (that is, nutrients). This pathway, together with the intensively secreted insulin after a meal, besides the synchronization of development and growth of the organism, constitutes one of the most important pathogenesis (additionally to DNA damage, telomere shortening, response to stress and chronic inflammation) involved in longevity [9]. IGF-1 is one of the main anabolic substances in the body. It affects the growth of cells, and by preventing apoptosis, it determines also the lifespan and stimulates cellular differentiation. Paradoxically, an increase of IGF-1 (e.g. stimulated by ingestion) does not prevent aging, but it is the reduction of the level of growth hormone and IGF-1 that facilitates longevity. It has been proven that mice with growth hormone deficiency live longer. A greater resistance to stress and increase in the synthesis of antioxidant enzymes has been observed among the mice. Although a complete suppression of the pathway dependent on IGF-1 proved to be lethal, the reduction of its activity results in prolongation of life in mice [10]. The genes that regulate the activity of a protein cascade involving IGF-1 and influencing the extension of life include, among others, *daf-2* (others are *age-1*, *age-2, akt-1),* which encodes the protein with a homonymous name. This peptide being evolutionary conservative, is the key regulator of the intracellular cascade involving IGF-1 [11]. The scientific reports concerning humans also points at few genes associated with the GH/IGF-1 axis and affecting the length of life. The greatest importance is attributed to a variant of *FOXO3A* gene that is more common in people older than 100 years. This gene is considered to be homologue to the *daf-16* gene that cooperates with *daf-2* in *Caenorhabditis elegans* [12]. Moreover, mutations of the receptor for IGF-1 are also noteworthy [32].

The pathway involving IGF-1 indirectly leads also to an increase in the activity of mTOR kinase. This protein from the group of serine-threonine kinases is stimulated by IGF-1 involving an intermediate molecule, i.e. phosphatidylinositol. mTOR kinase is one of the key intracellular factors participating in the modulation of the growth, proliferation and metabolism of a cell. It happens in response to signals from the activated membrane receptors. In addition, oxygen and/or energy substrates concentration in a cell is also important. The purpose of mTOR kinase is the phosphorylation of transcription factors responsible for the biosynthesis of the ribosomal protein. A pathological increase in its activity has been found in many diseases, in particular those related to cancer, and nowadays, it indirectly but strongly determines the lifespan [14]. In order to explain these phenomena, we have to go to the molecular level of a cell, at which mTOR kinase affects the cell cycle progress by biosynthesis of protein necessary for the production of cyclin D1. It causes transition of the cell from phase G1 to the cell division cycle S. Additionally, this type of kinase can also inhibit apoptosis. It happens by promoting the expression of protein being a member of apoptosis inhibitors family, the so-called survivin [15–17]. It is of vital importance that at the time of low energy level of the cell, the AMP-activated kinase (AMPK) stops the signalling depending on mTOR kinase. There is a hypothesis regarding many living organisms that the reduction of mTOR kinase activity may have a positive effect on the inhibition of cell proliferation and vitality [18]. This dependency was proved, among others, in the studies in heterogeneous mice. Starting from 600 day of life of animals, the inhibition of the pathway involving mTOR kinase by using rapamycin (mTOR kinase inhibitor) resulted in prolongation of their life by 9–14% [19].

Biochemically, the essential purpose of the caloric restriction is to reduce the activity of the signalling pathway involving IGF-1 and mTOR kinase activity. Reducing insulinemia and increasing insulin sensitivity of cells is intrinsically related to the reduction of calorie intake. In the studies by Roth et al., longer life among men with lower insulin concentration in the blood has been found. Moreover, a reduced transmission in the path involving IGF-1 is associated with reduced body weight and also reduced mortality among women [20, 21]. Apart from caloric restrictions, it is pointed out that the assessment of nourishment in terms of quality is also very important. Slim volunteers (average BMI 23.6 kg/m^2) from the association Calorie Restriction Society took part in the studies by Hollosza et al., believing that caloric restriction has the potential to extend life. A diet with a daily caloric restriction was recommended

to the volunteers who were observing it for 6.5 years. Furthermore, their diet was rich in nutrients exceeding even 100% of the recommend daily intake (vegetables, fruits, egg whites, lean meat, fish, grains, nuts). After completion of the research, it turned out that the average BMI of the participant was reduced to 19.6 kg/m^2 [22]. What is more, the risk of occurrence of diabetes mellitus type 2 among them, as well as atherosclerosis, left ventricular dysfunction and hypertension was comparatively low with the same risk documented in monkeys and rats that were subject to caloric restriction [23, 24].The same dependency was found in the context of biochemical parameters of blood in these volunteers (concentration of total cholesterol as well as LDL and HDL, glycaemia, insulinemia, CRP) compared to people who are on a typical American diet [24]. In the same year, the studies by Willcox and et al. were published. They concerned the influence of caloric restriction on health and lifespan of Japanese from Okinawa. This research group was of special interest because this island was occupied by the US (from World War II up to 1972). Therefore, the diet of its inhabitants included mainly plant-origin products and was low in meat and fish. I turned out that the total caloric value of this nourishment was lower by 11% than the diet of other Japanese. The results clearly showed that the average life expectancy of women and men from Okinawa is higher than of other inhabitants of the Land of the Rising Sun. Also the number of centenarians from this island is about 4 times higher than among the inhabitants of other well-developed countries. Moreover, the native Japanese from this island not only live longer but they also later and less likely fall for cancer and cardiac diseases [25].

Caloric restriction — imitation of this condition by metformin

Nowadays, the caloric restriction seems to be simple only theoretically. In order to achieve the above-mentioned results, it should consist of reduction of ca. 30–40% daily ration of food depending on gender and daily activities. To enhance the clinical effect of caloric restriction and/or to replace it, the substances which are to exert the effect similar to caloric restriction are used. Longo et al. pre-divided drugs with the potential to imitate this effect as to their mechanism of action. The first group relates to the mechanisms of action of drugs at the gene level, responsible for reduction of GH and IGF-1 secretion. Another group was to act by inhibiting the release of IGF-1 from hepatocyte. Furthermore, the substances that directly influence the signalling pathway IGF-1 and causing reduction of concentration of its final products or substances that mediate this process were also distinguished in the report [26, 27].

Table. 1. Metformin effect mitigating the changes at the molecular and tissue level occurring during aging

Parameters	Aging	Effects of metformin
ROS production	↑	↓
AGEs production	↑	↓
Activity of mTOR kinase	↑	↓
Gene *p53* mutations	↑	↓
Glucose tolerance	↓	↑
Insulinemia	↑	↓
LDL cholesterol concentration	↑	↓
Inflammation	↑	↓
Risk of cancer	↑	↓
Life expectancy	↓	↑

ROS — *reactive oxygen species*; AGEs — advanced glycation end products

From the pathophysiological point of view, metformin works as caloric restriction, resulting in the reduction of the energy state of the cell. Consequently, the ratio of AMP to ATP increases in the cell. This leads to the activation of AMPK which is considered as the energy "sensor" in the cell. Thus, the anabolic processes are inhibited, inclusive of gluconeogenesis. The profile of a liver cell switches to catabolic and opposite processes intensify, i.e. fatty acid oxidation (phosphorylation of acetyl-CoA) and glycolysis. The body weight of patients who take metformin does not increase, quite the contrary, some reports documents its reduction. The improvement of parameters of glycaemia, insulinemia and the parameters concerning lipid metabolism is one of the numerous beneficial effects of this drug. Increasingly reported reduced risk of cancer is another effect of the biguanide derivative. This and other effects of metformin which are counterweight to the aging process have been presented in Table 1 [28–31].

Research overview

One of the first reports concerning the role of biguanide derivatives as a geroprotector was presented about 40 years ago [32]. Currently, their action prolonging life was proved in numerous observations carried out on model animals. The study of Anisimov et al. carried out on female transgenic mice of HER-2/neu line showed that after application of metformin 100 mg/kg, the average lifespan of test animals extended by ca. 8–13% and a maximum of 1 month. What is more, a slower increase of glycaemia and triglycerides concentration in the blood, reduction of total cholesterol and β-lipoprotein compared to the control group

was observed within the test group [33]. A few years later, another study on mice of the same line was carried out under the guidance of the same author, and it showed a similar percentage of life extension in animals treated with metformin. Furthermore, in the context of anticancer potential of this drug, it was observed that breast adenoma occurs 10% later, and the use of metformin in combination with melatonin proved to reduce the growth of Ehrlich tumour (causing ascites in laboratory animals) by more than 40% [34]. The same author used female mice from an outbred line in another research, assessing the anti-aging potential of metformin. The animals from the research group taking metformin (100 mg/kg b.w.) reduced body weight, despite a slight reduction in food consumption. The average length of their life increased by more than 35% and the maximum lifespan increased by ca. 10% (by more than 2.5 month). The time in which mice were fertile has also been extended. Nevertheless, no anticancer role of metformin was found in this report [35].

In the context of the size of the anti-aging potential of metformin, the sex of animals proved to be also of vital importance. It has been proven that among female mice of 129/Sv line, a chronic therapy with metformin extended their lives by ca. 5%. Such an effect has not been observed among males of the species, quite the contrary, the average of their lifespan has been reduced by more than 10%. The same occurs in terms of the anti-cancer role of metformin. The incidence of malignant tumours has reduced 3.5 times in females, and it has not been observed in males of this species [36]. The purpose of another research was to answer the question, when does the commencement of therapy with metformin determine the lifespan to the greatest extent. For this purpose, the age of animals at which the therapy with this drug was commenced was evaluated. The research group consisted of 3 groups. In the first group, metformin was started to be given to mice at the age of three months. The second group commenced the therapy after reaching the age of 9 months, and the last one at the age of 1 year and 3 months. As it turned out, the therapy with metformin delayed disruption of reproductive functions in each of these groups. Additionally, this therapy did not contributed significantly to the change of concentration of cholesterol and glycaemia in none of the groups. With regard to the anti-aging effect, it was shown in the study that the earlier the therapy with metformin is started, the greater potential to extend life is achieved (14% and 6% in the first and second group, respectively). In the third group, in which the metformin therapy was started at the latest, no prolongation of life under the influence of this drug has been observed. Retardation of development of cancer diseases was noticed also only in two first groups [37]. Some of the latest reports of Anisimov et al. constitutes, to some extent, a continuation of the previous one. According to the hypothesis of the author, the perinatal age constitutes the major determinant in the development of hypothalamic mechanisms controlling the energy homeostasis. Following this reasoning, thanks to the mechanism of action of metformin (that is incorporated into the therapy at that time) it is possible to delay the onset of age-related diseases. In the study, metformin was administered to the male and female mice of line 129/Sv, SC on 3rd, 5th and 7th day after birth. The results show that the amount of food eaten by male decreased and their lower body weight (compared to the control group) has maintained for the entire life. It is also interesting, that the average lifespan of male receiving metformin extended by about 20%, and the maximum lifetime by 3.5%. No such effect has been observed in female. Their average life expectancy decreased by ca. 14% in the research group. The endpoint of the study was reaching the age of 800 days, and almost half male of the control group and nearly 75% of the research group lived till that time. Slightly more than 50% of female mice of the control group and only 1/3 of the research group lived till that time [38]. The results of the research carried out by the Research Team from the National Institute on Ageing, Baltimore (USA) present the correlation of the applied metformin dose on the size of the effect prolonging life. After the animals approach the middle-age (600 days), metformin was administered to them in concentration of 0.1% and 1% of the diet weight fraction. It was found after several months of the study that males taking a lower dose of the drug lived longer by 5% than mice that did not receive the drug. Moreover, they also characterized with a lower frequency of incidence of circulatory system disorders. Conversely is with the mice that were given a higher dose of metformin. They were dying faster than mice from both groups subject to the research. According to the authors of the study, these dependencies can be explained with the claim that metformin applied in low doses mobilizes the body to work more efficiently with a reduced amount of produced reactive oxygen species that damage cells and tissues of the body [39].

The report of Onken et al. based on *C. elegans* documents also the relationship between the metformin dose taken and the achieved different length of life. A slight increase of the maximum life expectancy with a strong influence on the average age of these nematodes was achieved with the application of only 50 mmol/l of metformin. The desired effects with ap-

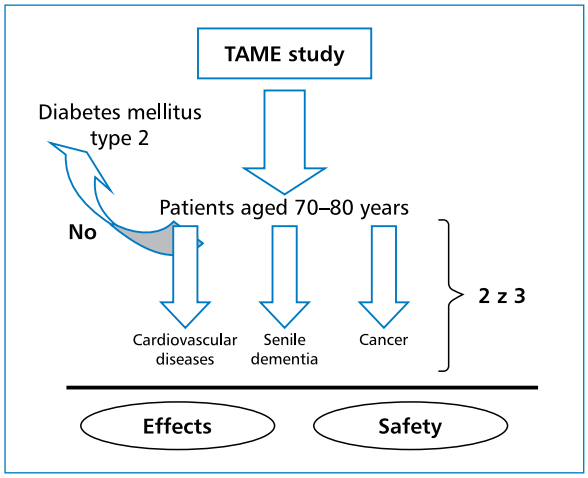

Figure 1. TAME research — main assumptions

plication of a lower dose (10 mmol/l) and a greater dose (100 mmol/l) were negligible [40].

Taking the abovementioned issues into account, among the clinical tests those carried out by Bannister et al. are noteworthy. The report showed that patients with diabetes mellitus type 2 and regularly receiving metformin in the monotherapy characterize with even more than 15% longer life than people without diabetes and who do not take metformin. This peculiar paradox observed in this study strongly encouraged scientists to determine the potential of metformin to prolong lives of people [41].

The hopes of clinicians in determining the anti-aging role of metformin in the strict sense is associated with the recently launched TAME study (Targeting Aging with Metformin). The study that is financed by the American Federation for Aging Research is spearheaded by Dr. Barzilai. A double-blind, placebo-control study will be the first such clinical and scientific trial determining the anti-aging potential of metformin. Its premise is to administer this drug to 3000 patients who are between the 7th and 8th decade of life. Only the patients who suffer one or two of these three age-related diseases, i.e. heart diseases, malignant tumour or cognitive impairment, may take part in the research. The study is intended to answer the question, whether metformin has the ability to inhibit the progress of such diseases and delay mortality associated with these disorders. It is significant in this research that the patients with diabetes mellitus type 2 are not qualified to participate, because the assumption of the authors is that presumably, they have been treated with metformin for an indefinite period of time. Consequently, it could bring inadequate results of this study. What is more, the study also aims to answer the question, whether a long-term treatment with metformin of patients without diabetes mellitus type 2 is safe (Fig. 1) [42–44].

Summary

Metformin is one of the most widely prescribed drug in the world. Pharmacies receive more than 120 million prescriptions for this drug yearly [45]. Undoubtedly, it has a pleiotropic effect in the body and it is advisable to search for its new applications. One of them is the anti-aging activity of metformin. Successful results of animal research induce the scientists to boldly apply these effects also to the people. However, clinical research and more extensive data on the subject are required to confirm the above. The authors of the study are eagerly waiting for the results of the TAME project which can be somehow the beginning of an avalanche of studies concerning the anti-aging potential of metformin among the people. The biochemical basis for metformin effect and its imitation of caloric restriction, which in itself has an undeniable anti-aging effects, may justify that this drug can in a well-known manner logically explain the anti-aging effect.

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