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Minireview

Metformin: Prevention of genomic instability and cancer: A review

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ABSTRACT

The diabetes drug metformin can mitigate the genotoxic effects of cytotoxic agents and has been proposed to prevent or even cure certain cancers. Metformin reduces DNA damage by mechanisms that are only incompletely understood. Metformin scavenges free radicals, including reactive oxygen species and nitric oxide, which are produced by genotoxicants such as ionizing or non-ionizing radiation, heavy metals, and chemotherapeutic agents. The drug may also increase the activities of antioxidant enzymes and inhibit NADPH oxidase, cyclooxygenase-2, and inducible nitric oxide synthase, thereby limiting macrophage recruitment and inflammatory responses. Metformin stimulates the DNA damage response (DDR) in the homologous end-joining, homologous recombination, and nucleotide excision repair pathways. This review focuses on the protective properties of metformin against genomic instability.

1. Introduction

Genomic instability is an abnormal increase in mutations to the genome, which can be transferred to offspring cells. Cytotoxic agents, such as non-ionizing and ionizing radiation, free radicals, and metals, attack the genome thorough mechanisms that include generation of reactive oxygen species (ROS) and damage to nuclear or mitochondrial DNA, cell membranes, and enzymes [1]. These processes contribute to genomic instability, increasing mutation rates and resulting in the development of aggressive phenotypes and cancer. DNA damage may also lead to changes in the activities of genes involved in DNA repair, cell division, oncogenes, and tumor suppressor genes. Genomic instability is associated with increased mutation rate in genomic DNA; this may result from chronic ROS production, inhibition of antioxidant system enzymes, inflammation, and/or epigenetic changes [123–127].

Genomic instability has been examined by several endpoints, including chromosome rearrangements and aberrations, gene amplification, aneuploidy, micronuclei (MN) formation, microsatellite instability, and gene mutations [2]. Many compounds may mitigate genomic instability direct scavenging of ROS, hydrogen donation to reactive free radicals, inducing and/or altering the levels of endogenous enzymes for detoxifying ROS, and enhancing the DNA damage repair pathway [3,4].

Metformin is prescribed to over 120 million patients worldwide as treatment of choice for type 2 diabetes. The drug is considered safe, as its glucose-lowering actions are not accompanied by hypoglycaemia [5]. Metformin has many biological effects, including anti-inflammatory, anti-apoptotic, anticancer, hepatoprotective, cardioprotective, otoprotective, renoprotective, radioprotective and radiosensitizing, and antioxidant activities [6–15].

Several processes are implicated in the preventive action of metformin against genomic instability. One mechanism is protection against oxidative stress [16]. Metformin scavenges free radicals, prevents damage to nuclear and mitochondrial DNA, and enhances DNA repair [17]. In this article, we discuss the evidence for protective effects of metformin against DNA damage and cancer caused by genotoxic agents, and consider possible mechanisms for these effects.

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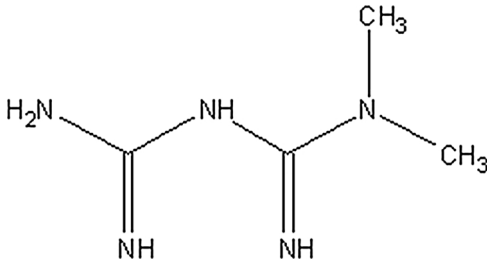

Fig. 1. Chemical structure of metformin (CAS 1115-70-4).

2. Metformin

Metformin (Fig. 1) is a biguanide derived from the perennial plant, *Galega officinalis* (French Lilac, also known as Goat's Rue, Italian Fitch, or Professor weed). Its medicinal use dates back to ancient Egypt and medieval Europe; tea infusions were used to treat polyuria and halitosis, both of which are now recognised as symptoms of diabetes [18]. In the 1920s, guanidine was identified as the active component of galega, and was used to synthesize several anti-diabetic compounds; in the late 1950s, French scientists linked two guanidine rings together, producing better tolerated anti-diabetic agents, metformin and phenformin. Metformin was approved for the treatment of hyperglycemia in Britain (1958), Canada (1972), and the USA (1995) [19–21]. In the 1970s, phenformin and buformin, more potent, lipophilic biguanides, were withdrawn from the market, due to an adverse reaction, lactic acidosis; this led to increased use of metformin [18–21]. Metformin has remained the first-line therapeutic option for type two diabetes, with approximately 120 million patients taking the drug, world-wide [22].

The therapeutic plasma level of metformin is 0.5–2.5 mg/l (3–15 μM) [23]. Oral bioavailability is 50–60%; absorption takes place from the small intestine; 90% of the dose is eliminated unchanged in the urine in 12 h [20]. Metformin does not bind to plasma proteins. Its plasma half-life in humans is 6.2 h, with maximum plasma concentration, 1–2 mg/l, reached 1–2 h after an oral dose of 500–1000 mg [20]. Currently, the approved dose of metformin is 1–2.55 g daily, administered twice daily, for a 60 kg patient.

The most frequent side effects associated with metformin are gastrointestinal, with over half of patients able to tolerate the maximum daily dose; however, it has been reported that 5% of patients are unable to tolerate any dose. The rare event of lactic acidosis occurs in approximately 3 per 100,000 patients and appears to be linked to renal insufficiency, impairing clearance and resulting in extremely high plasma levels of the drug. Thus, metformin is contraindicated in patients with substantial renal dysfunction. Anaemia due to vitamin B_{12} malabsorption and deficiency is also noted as a rare event [20,24].

3. Search strategy

We carried out a comprehensive search of Thomson ISI's Web of Science, MEDLINE (PubMed), Google Scholar, and Scopus databases for articles published from Jan. 1990 to May 2017, using the search term "metformin" combined with the terms "DNA damage", "DNA repair", "genotoxicity", " reactive oxygen species", "antioxidant", "cellular respiration", and "inflammation". We reviewed published articles and the bibliographies of selected manuscripts. The abstracts/titles of all articles identified by electronic searches were screened to determine whether they met the following inclusion criteria: (a) full abstract available online; (b) manuscript written in English; (c) focussed on or including the protective effect of metformin against DNA damage; (d) focussed on DNA repair by metformin; (e) focussed on antioxidant effects of metformin; (f) focussed on immunomodulatory activities of metformin; (g) focussed on anti-inflammatory effects of metformin; (h) focussed on epigenetic modifications by metformin; (i) focussed on anticancer effects of metformin.

4. Mechanisms of the protective effect of metformin

4.1. Metformin and cellular metabolism

4.1.1. Metformin and ROS

Metformin's protective effects have been have associated with oxidative stress, DNA damage and DNA damage repair; however, there is no consensus concerning the role of the drug. Several studies have shown that metformin reduces ROS generation induced by stressors, thereby protecting cells and ameliorating genomic instability and possibly cancer risk [16]. Metformin can detoxify ROS as a direct or indirect free radical scavenger, through donation of a H atom from its CH_3 or NH groups, and by up-regulation of thioredoxin activity [25–28]. The ability of metformin to counteract oxidative damage was confirmed in several experiments [29–31]. Metformin can reduce the level of intracellular ROS and γH_2AX or Ataxia Telangiectasia Mutated (ATM) protein kinase activation in normal mitogenically stimulated lymphocytes [32]. It decreases insulin-induced intracellular ROS production and DNA damage in normal rat kidney epithelial cells [33]. In diabetic animals, by decreasing oxidative stress, metformin reduces the number of micronucleated erythrocytes [34]; micronuclei (MN) are an indicator of genome instability [35].

We have shown that metformin does not cause DNA damage in human blood lymphocytes [36] *in vitro*. Our results are in agreement with those of Sant'Anna et al. [37]. Other studies showed non-genotoxicity of metformin in rat and mouse bone marrow cells [12,38].

In contrast, other studies have reported that metformin causes increased DNA damage signalling, *i.e*, increased γH2AX expression in hepatoma cells [130] and increased γH2AX focus formation in pancreatic cancer cells [131]. These studies hypothesized that DNA-damaging effects of metformin are due to diminished DNA repair resulting from ATP depletion [130] or metformin-induced AMPK-dependent activation and consequent downregulation of the mTOR signaling pathway [131].

Onarn et al. found that metformin, at pharmacological concentrations, has no modifying effect on chemically induced DNA damage in cultured human lymphocytes, despite its partial protective effect against lipid peroxidation. However, higher metformin concentrations increased cumene hydroperoxide (CumOOH)-induced DNA damage [46].

Amador et al. reported that chronic treatment of Chinese hamster ovary (CHO-K1) cells with metformin may be genotoxic [39]. The concentrations of metformin used in these two studies (114.4 and 572 μg/ml, respectively) are 40–170-fold greater than the recommended therapeutic plasma level [40]. Harishankar et al. compared type 2 diabetes patients receiving metformin to healthy controls, and reported increased MN frequencies in the patients; however, type 2 diabetes can cause oxidative stress that could elevate MN frequencies [41].

Disagreements in the literature regarding metformin and DNA damage demonstrate the need for further investigation. Both genetic and environmental factors are associated with genotoxicity [41]. Table 1 summarizes the protective effects of metformin against genotoxicity induced by exogenous and endogenous agents. Fig. 2 illustrates mechanisms of action of metformin in response to toxicant-induced genomic instability. Fig. 3 illustrates possible molecular mechanisms for the protective effects of metformin.

4.1.2. Metformin and the antioxidant system

Metformin activate endogenous repair systems, preventing ROS toxicity. The drug enhances the activity of the AMPK pathway and increases the expression of thioredoxin through the forkhead transcription factor 3 and TRX functions [30,42]. The consequent decrease in ROS levels may reduce genomic instability and possibly cancer risk [16,43,44].

Administration of metformin to rats decreased frequencies of MN