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Original Research Article

Investigation of the Chemistry of Metformin by Targeting the Nrf2 Signaling Pathway (A response Surface Methodology Approach)

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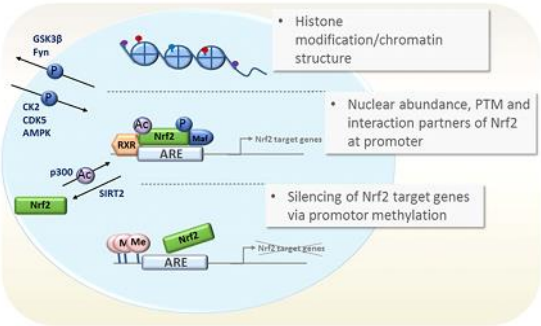
ABSTRACT

According to previous chemistry researches, Nrf2 is an essential leucine zipper protein that can regulate the expression of antioxidant proteins and protect against oxidative damage caused by inflammation or injury. Nrf2 effectively activates gene products, thereby reducing reactive oxygen species and electrophiles; therefore, it can prevent the progression of many chronic diseases or delay them. It is also effective in regulating several metabolic genes (purine nucleoside biosynthesis, pentose phosphate pathway, and increasing mitochondrial function and fatty acid metabolism). The balance between oxidation and antioxidants in the body is called oxidative stress. Superoxide dismutase and glutathione peroxidase, which are endogenous antioxidants, cannot counteract the oxidative regulation of lipids, proteins, DNA, and cellular structures in oxidative stress. During diabetes, Nrf2 expression decreases, thus accelerating and intensifying the oxidative stress process. It can also be suggested that the decreased Nrf2 expression and increased oxidative stress in kidney tissue can affect the number of proteins involved in apoptosis, such as Bax and BCL2 affect and accelerate the process of apoptosis in kidney cells. pacificus bacteria as a new species that can bioremediation.

GRAPHICAL ABSTRACT



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Introduction

Diabetes mellitus is characterized by high blood sugar due to abnormal insulin secretion or insulin dysfunction and is a metabolic disorder. Its prevalence is currently increasing sharply [1]. High blood sugar in diabetic patients can create conditions for oxidative stress [2]. Oxidative stress induced by hyperglycemia can lead to diabetic nephropathy by activating the apoptotic process, the most common microvascular complication [3,4]. One of the proteins that play a central role in the defense against oxidative stress is the transcription factor Nuclear factor-erythroid 2 related factor 2 (Nrf2), Which can bind to the antioxidant response element (ARE) and increase the body's antioxidant defense by expressing effective enzymes [5,6].

The first line of treatment in diabetes is hypoglycemia, and metformin (1, 1-dimethylbiguanide hydrochloride, MET) is a common drug used for this purpose, has Mitotic inhibitor, an Angiogenesis inhibitor, and anti-inflammatory properties. In addition, it may have beneficial effects on Nrf2 expression, ultimately reducing oxidative stress and the process of apoptosis and improving the course of diabetic nephropathy, which occurs in people with diabetes [7,8]. However, this drug has side effects. In addition, it is thought that despite the beneficial effects of MET on the mentioned pathways that are effective in the increased diabetic nephropathy [9]. Metformin has not been very successful in preventing diabetic nephropathy [10,11]. Despite treating people with diabetes with this drug, (Figure 1), the number of people with diabetic nephropathy is increasing [12,13].

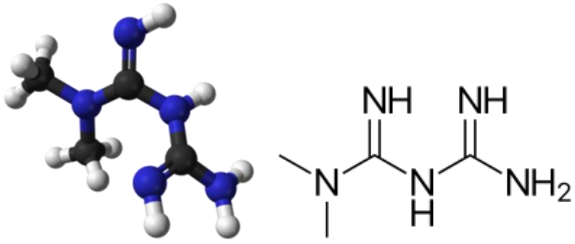

Figure 1: Chemical structure of Metformin ($C_4H_{11}N_5$)

It has been used extensively in the clinic for the past 60 years, and its effectiveness and safety have been proven [14]. MET is effective in reducing serum triglyceride levels, serum low-density lipoprotein cholesterol (LDLC), and increasing serum high-density lipoproteins (HDL) level [15] (Figure 2).

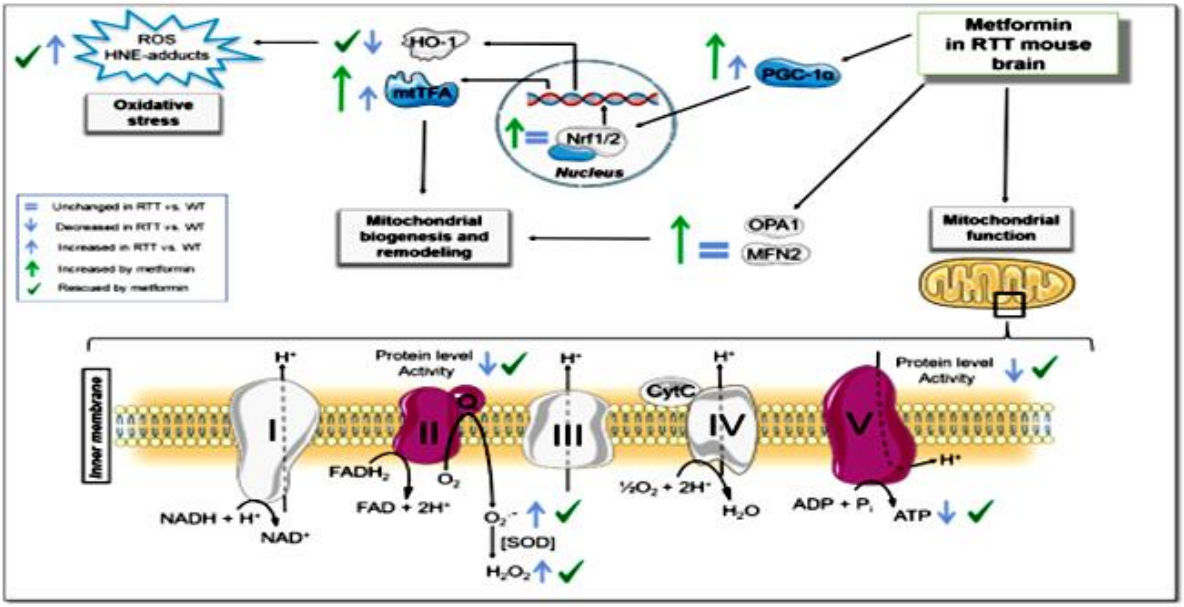

Figure 2: Metformin activates the nuclear respiratory factor 2 (Nrf_2) (mouse model)

MET exerts its effect by activating the protein kinase activated by 5 'adenosine monophosphate (AMPK), reducing gluconogenesis in the liver, and improve insulin binding to muscle and liver receptors. As a result, it increases glucose uptake and insulin sensitivity [16].

Studies have reported that MET can suppress inflammation and activate Nrf2 pathways in neurons [17].

Neuroprotective Effects of MET in Neurological Disorders

Patients with D2M are at greater risk for Alzheimer's disease (AD) [18]. AD is a neurodegenerative disease that destroys the gray matter cells of the brain. The cause of this progressive disease is the deposition of a substance called beta-amyloid (Aβ) [19]. Studies revealed that antioxidants may delay the progression of AD [20]. Allard *et al*., 2016 [21] evaluated that MET may attenuate CNS-based inflammation in older C57BL/6J mice and reported that MET decreased transcription and activated the Nrf2. MET reduces α-synuclein phosphorylation, mitochondrial disorders, and oxidative stress [22]; as a result, MET have antioxidant properties [23]. Allard *et al*., [21] in Long-term metformin therapy, Nrf2 can reduce expression and activation and increase longevity by increasing Nrf2 levels [24].

Markowicz-Piasecka *et al*., [25] showed that MET could be an effective treatment in preventing AD and other neurodegenerative diseases with its multifaceted properties and safety pharmacokinetic characteristics. In a vivo study by Oliveira *et al*., [26], the result showed anti-inflammatory properties of MET such as Reduction in expression of the astrocyte and microglial markers, inflammation markers (IkappaB proteins, Vascular endothelial growth factor (VEGF)), interleukin 1, and Simultaneous increase in p-AMPK and Nitric oxide synthases (NOSs).

Chin-Hsiao *et al*., [27] reported MET use is associated with reduced dementia risk. Prasad *et al*., [28] provide evidence of an association between smoking, induced cerebrovascular/ blood-brain barrier (BBB) impairments with Nrf2 And that these effects can be prevented with MET. Also, there should be more discussion about Nrf2 and AMPK and their interactions. According to Arefin et al., study [29] Nrf2 is a significant and direct regulator of antioxidant responses, and oxidative stress is also known to cause cellular aging so that Nrf2 can prevent premature aging of arteries and calcification (Figure 3).

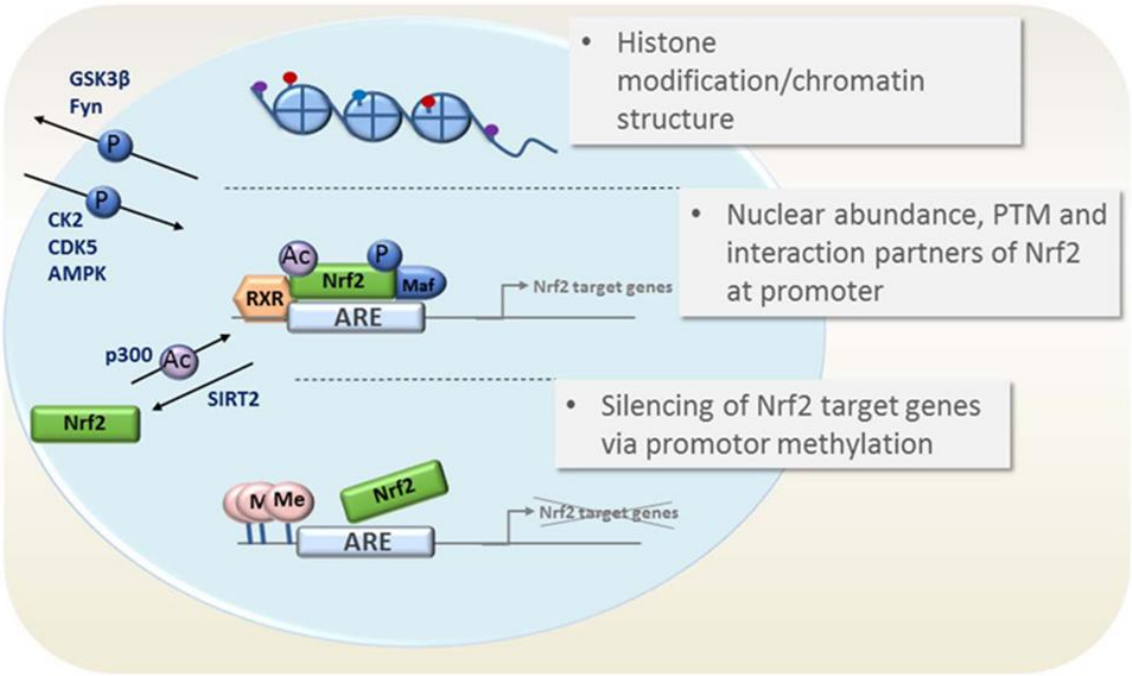

Figure 3: Transcription controlled and Transactivation genes regulated by Nrf_2

Studies have shown that MET can prevent neurological disorders. Gantois *et al*., [30] assessed the influence of MET for the treatment of Fragile X Syndrome, and the result showed positive effects of MET.

Nandini *et al*., [31] showed MET could play a protective role against diseases such as Parkinson's, stroke, Huntington's disease, and epilepsy. Peralta *et al*.,2020 [32] reported MET in a mouse model with neuronal complex I delay the onset of neurological symptoms.

Zhao *et al*.,2020 [33] The effect of MET on the protection of RPE cells in H2O2-induced oxidative damage was investigated. The results showed that metformin could have a protective role by activating Nrf2 [34].

Kim *et al*., [35] reported MET exerts beneficial effects on hemorrhagic shock independently of AMPKα1. Zhang *et al*.,2013 [36] Showed that the potential use of Nrf2 inducers in the treatment of neurological diseases can have a protective effect, and long-term treatment with metformin can reduce Nrf2 transcription [21].

One study reported that MET could reduce the incidence of PD in patients with type 2 diabetes [37]. It can also delay permeable passage pores' opening by preventing the early apoptosis of cortical nerve cells [38]. MTF also increased Bcl-2, decreased Bax expression, and decreased caspase-9 activation [39].

El-Ghaiesh *et al*. [40] indicated that MET improved animals' motor function and Nrf2, rotenone mouse model.

Nrf_2 can improve memory performance in the hippocampus of the ischemia model in mice and protect eNOS / Nrf_2 / HO-1 Cell signaling against ischemic neuronal injuries [41]. Ashabi, G *et al*.,[42] showed that MET can protect cells by inhibiting inflammation and inducing Nrf_2 antioxidant pathways.

Cardiovascular and respiratory protective effect

Pioglitazone, an insulin-sensitive agent, has been reported to affect cardiovascular oxidative stress in sucrose-induced metabolic syndrome (MetS). On the other hand, thyrodoxin (TRX) can play anessential role in the fight against oxidative stress and cardiovascular disease.[43].

Nrf2 effectively treats diabetic complications such as cardiovascular disease and diabetic nephropathy. In Wu *et al*.,2016 [44] study, the potential modulatory effects of Nrf2 were investigated. NAD (P) was associated with H dehydrogenase (quinone) 1 (NQO-1).

Hasanpour Dehkordi *et al*., 2019 [45] evaluated the role of metformin in inhibiting inflammatory processes and oxidative stress. The study results showed that the mechanism of action of this drug has been different in different conditions. The Nrf2 pathway plays an essentialessential role in the drug resistance of adenocarcinoma and can act through various mechanisms (mitogen-activated protein kinase pathway). The effect of metformin on Nrf2 and Nrf2 / ARE regulation in adenocarcinoma.

Hepatoprotective effect, Reno-protective effects, Skin protective effect and chemoprotective effect

Clinical studies show diabetes patients taking metformin reduced their risk of cancer. Metformin can activate AMPK by inhibiting mitochondrial I complex I, inhibiting mitogen-activated protein kinase (MAPK), inhibiting the phosphorylation of Smads, and protecting the liver against chemical or viral hepatic toxins [46]. Urpilainen *et al*., 2019 [47] Expression of high cytoplasmic Nrf_2 levels has been reported to predict unpleasant overall survival and specific survival of breast cancer. Metformin has been reported to have a protective effect against lead toxicity and act as an inhibitor of mitochondrial fragmentation, reducing lead-induced cell damage, Yang *et al*., 2020 [48].

Results and Discussion

In recent years, Nrf_2b in the prevention and treatment of cancer has been investigated. It seems that Nrf_2 activation and thus increase cellular detoxification response protects against cancer. Role of Nrf_2, especially in diabetic patients with cancer, has been challenging to address so far and deserves future attention., Matzinger *et al*., 2018 [49].

The NRF_2 / KEAP1 pathway is one of the most important cellular defense mechanisms against stressors (internal and external). NRF_2 transcription factor can protect cells and tissues

from damage, oxidative stress by increasing the expression of several cellular protection genes.

Studies have reported that NRF_2 is an effective cancer treatment. Numerous studies have been performed to identify treatment strategies and investigate the role of NRF_2 [50]. In Panieri *et al*.,2019 [51] studied the use of some Nrf_2 inhibitors in treating cancers.

The findings suggested that NRF_2 could protect against cancer malignancy and treatment resistance by controlling intracellular redox homeostasis by activating cellular protective antioxidant genes.

Numerous studies are being conducted to evaluate the effect of metformin alone and combination with other drugs in cancer treatment. Metformin induces both AMPK-dependent and AMPK-independent genes/pathwayscancer cells' growth and migration, inhibiting cancer cells' growth and migration, and inducing apoptosis, Safe *et al*., 2018 [52].

Metformin can reduce the expression of Sp1, Sp3, and Sp4 genes and the precancerous regulator Sp 82; one of the mechanisms is to target Sp transcription factors that make metformin successful [53]. These reports are consistent with the effects of metformin on genes/pathways in many other types of tumors [52].

Kamarudin *et al*.,2019 [54] in different models of colon cancer, they were able to study the effects of metformin. This study observed that metformin has an influential role in cell cycle, apoptosis, oxidative stress, epigenetic regulation, and inflammation.

Anti-inflammatory and anticancer activity and cell survival can be affected by maintaining redox homeostasis [55-57]. In the last decade, conflicting evidence has been reported regarding the effect of Nrf_2 on cancer [58-60] and poor prognosis in abnormal Nrf_2 activation [61-63]. Nrf_2 can play a role in developing pre-survival genes in most cancers, causing cancer cells to proliferate by metabolic reprogramming, apoptosis by suppressing cancer cells, and increasing the capacity for self-renewal of cancer stem cells. There is ample evidence that Nrf_2 contributes to cells' chemotherapy and radiological resistance. A new strategy targeting Nrf_2 in the treatment of cancer patients is a promising therapeutic approach., Wu *et al*., 2019 [64-66].

Conclusion

Overall, the role of Nrf_2 pathway had a critical function in the modulation of oxidative stress, inflammatory responses. As a result, it was found that regulation of Nrf_2 is necessary to prevent the progression of several pathological diseases and is also effective in maintaining cellular homeostasis. The present study focused on the effect of metformin on Nrf_2 signaling pathways. The findings demonstrated that Nrf_2 pathway activation can effectively treat neuroprotective, neuroprotective, cardioprotective, renoprotective, hepatoprotective, and anti-tumor, which metformin activates Nrf_2 transcriptional activity.

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Authors' contributions

All authors contributed toward data analysis, drafting and revising the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

We have no conflicts of interest to disclose.

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