Oceana Biomedicina Journal OBJ
ocean-biomedicina.hangtuah.ac.id/index.php/journal
eISSN 2614-0519
Volume 7 Issue 1: January – June 2024
RESEARCH STUDY

The Role of Metformin on the Expression of Apoptosis-Related Caspase Family Members

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ABSTRACT

Metformin is the main line drug in the treatment of type 2 diabetes mellitus. However, now metformin has been widely studied for its potential for the treatment of various diseases. Apoptosis is a programmed cell death mechanism mediated by various proteins from the caspase family. Furthermore, the apoptotic caspases are subdivided into the initiators (caspase-2, -8, -9, and -10) and the effectors (caspase-3, -6, and -7). Various studies have reported that metformin could induce apoptosis, while other studies have reported otherwise. This study aimed to reveal the role of metformin on the expression of caspase proteins associated with apoptosis. Bioinformatics analysis using STITCH v.5.0 was performed to predict the metformin-caspase interactions. The results showed that metformin could directly activate caspase-3 and -7. However, at the same time, metformin could also inhibit the expression of caspase-3 and -8. Furthermore, metformin did not interact directly with caspases -2, -6, -9, and -10. Through this study, it can be concluded that metformin has dual roles (activation and inhibition) in the expression of apoptosis-related caspase proteins.

Keywords: Apoptosis, bioinformatics, caspase, metformin, STITCH.

1. Introduction

Metformin is a widely used biguanide drug due to its safety and low cost. It has been used for over 60 years to treat type 2 diabetes at the early stages because of its outstanding ability to decrease plasma glucose levels. Over time, different uses of metformin were discovered, and the benefits of metformin for various diseases and even aging were verified. These diseases include cancers (e.g., breast cancer, endometrial cancer, bone cancer, colorectal cancer, and melanoma), obesity, liver

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diseases, cardiovascular disease, and renal diseases. Metformin exerts different effects through different signaling pathways [1].

In recent years, many additional unexpected but effective roles of metformin were found. Studies showed that metformin exerts a strong effect on numerous cancers [2,3], cardiovascular disease (CVD) [4], liver diseases [5], obesity [6], neurodegenerative diseases [7], and renal diseases [8]. Sole medication or combination therapy with other drugs has been shown to be effective to treat different diseases [1].

Cell death is a fundamental process that maintains tissue homeostasis, removes unwanted or damaged cells, and ensures the recycling of cellular constituents promoting further growth and differentiation [9]. Apoptosis is involved in aging and age-related disease, with respect to aging, apoptosis acts in a cell type-specific manner. The rate of apoptosis is elevated in most types of aging cell populations and organs. In stable cells and certain continuously dividing cells, apoptosis serves to eliminate presumably dysfunctional cells that show homeostatic failure due to oxidative stress, glycation, and DNA damage, thereby maintaining homeostasis in the body [10]. Caspases (cysteine-aspartic proteases) are proteolytic enzymes largely known for their role in controlling cell death and inflammation. Based on their function, mammalian caspase-2, -3, -7, -8, -9, and -10 are apoptotic caspases, whereas caspase-1, -4, -5, -11, and -12 are involved in inflammation. The apoptotic caspases are subdivided into the initiators (caspase 2, 8, 9, 10) and the effectors (caspase 3, 6, and 7) [9].

Metformin has a very controversial role in apoptosis. Accumulating evidence indicates that metformin inhibits the growth, survival, and metastasis of different types of tumor cells, including those from breast, liver, bone, pancreas, endometrial, colorectal, kidney, and lung cancers [11]. A study by Jang *et al.* (2018) showed that degradation of cellular caspase 8 (FLICE)-like inhibitory protein (c-FLIP) and activation of procaspase-8 were associated with metformin-mediated apoptosis [12]. Queiroz *et al.* (2014) also reported that in MCF-7 cells metformin decreased the activation of IRβ, Akt, and ERK1/2,

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increased p-AMPK, FOXO3a, p27, Bax, and cleaved caspase-3, and decreased phosphorylation of p70S6K and Bcl-2 protein expression [13]. The administration of metformin with various doses (5, 10, and 50 μ M) of various duration (24, 48, and 72 h), could increase the expression of caspase-8 and caspase-9 mRNA in the T47D breast cancer cell line [14]. However, some studies showed that metformin could decrease some proteins related to apoptosis. Metformin treatment significantly inhibited the expression of Bcl-10, Bid, and caspase-3 in peripheral blood mononuclear cells in type II diabetes mellitus [15]. Chen *et al.* (2016) that found metformin could protect against apoptosis and senescence in nucleus pulposus cells and ameliorates disc degeneration *in vivo* [16]. Furthermore, metformin has been identified to promote proliferation and suppresses apoptosis in Ox-LDL-stimulated macrophages by regulating the miR-34a/Bcl2 axis [17]. Metformin was also reported to inhibit Aβ 25-35 -induced apoptotic cell death in SH-SY5Y cells [18].

Based on some of the evidence above, it can be identified that metformin can act controversially as both antiapoptotic and proapoptotic. However, the molecular mechanism remains unanswered. This study aims to reveal the role of metformin on the apoptotic caspase initiators as well as the executioner.

2. Materials and Methods

This study used a bioinformatics approach to reveal the molecular action of metformin on the caspase family associated with apoptosis. The analysis was carried out using STITCH software version 5.0 (http://stitch.embl.de/). The keywords or identifiers used are "metformin" as the drug to be analyzed followed by the apoptosis protein initiators "casp2", "casp8", "casp9", and "casp10". Then, for executioner apoptosis, "casp3", "casp6", and "casp7" were used. Furthermore, in the organism selection section, *Homo sapiens* was selected for analysis because we focused on molecular pathways in humans. STITCH

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v.5.0 predicted the molecular interaction between metformin and caspase family proteins by generating a signaling pathway map.

3. Results and discussions

In this research, the interaction of metformin and four proteins of apoptosis initiator (caspase 2, 8, 9, and 10) and three proteins of apoptosis executioner (caspase 3, 6, and 7) were analyzed by using STITCH v.5.0 as shown in Figure 1. Moreover, the molecular interactions between metformin and apoptosis initiator and executioner are summarized in Table 2. Metformin has direct interaction with the initiator caspase 8, executioner caspase 3, and caspase 7. While did not have direct interaction with initiator caspase 2, 9, 10 and executioner caspase 6.

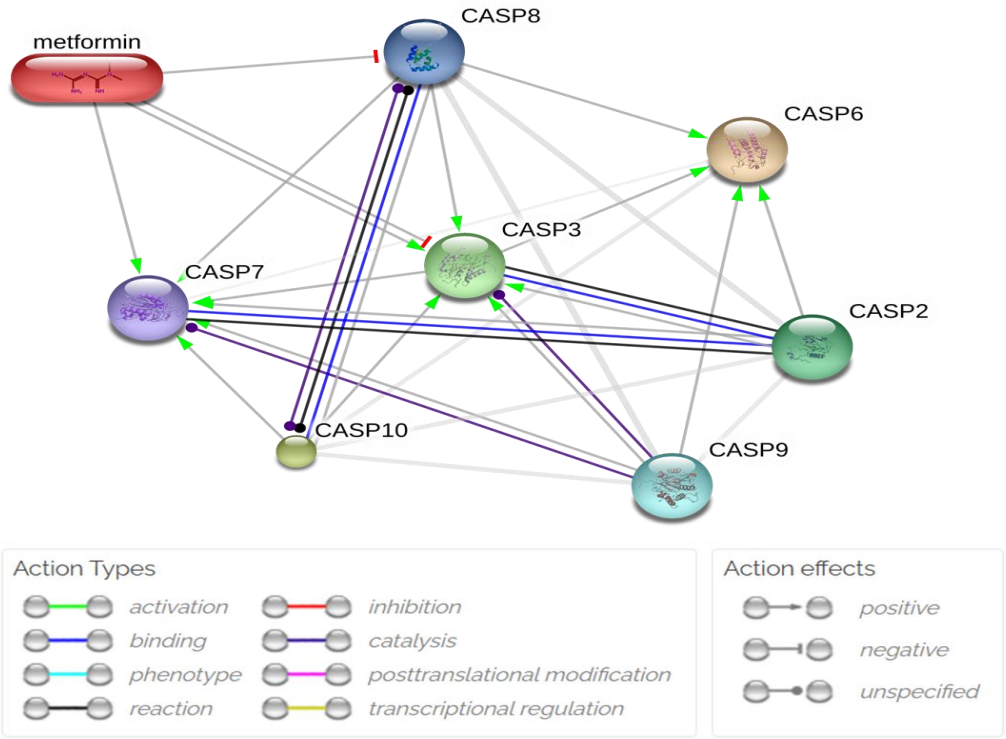

Figure 1 Molecular interaction between metformin and apoptosis-related caspase family members analyzed by STITCH v.5.0.

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Table 2 Molecular interaction summary of metformin and caspases

Drug	Protein / Type of caspase	Molecular action
Metformin	Caspase 2 / Initiator	None
	Caspase 8 / Initiator	Inhibition
	Caspase 9 / Initiator	None
	Caspase 10 / Initiator	None
	Caspase 3 / Executioner	Activation and inhibition
	Caspase 6 / Executioner	None
	Caspase 7 / Executioner	Activation

3.1 Interaction of metformin with caspase 8

The interaction with the initiator caspase 8 was inhibition. Caspase 8 is one of the central roles in apoptosis and modulates inflammation. In apoptosis, activation of caspase 8 was the result of the death receptors and ligand engagement such as Fas (CD95), TNF-related apoptosis-inducing ligand (TRAIL), and TNF receptor 1 (TNFR1). The caspase 8 adaptor Fas-associated protein with death domain (FADD) initiates an apoptosis cascade that leads to the induction of executioner caspase-3 (extrinsic)-dependent and caspase-9 (intrinsic)-dependent pathways [19]. Although caspase 8 modulates apoptosis, the limiting step for caspase-8-dependent pro-apoptotic activity is related to the presence of the short segment of c-FLIP. When the levels of c-FLIP are high, pro-caspase-8 preferentially heterodimerizes with it leading to an insufficient amount of active caspase-8 to induce apoptosis, so favoring cell survival and proliferation [19,20]. Thus, the inhibition of caspase-8 reduced the cancer cell outgrowth.

In inflammation modulation, caspase-8 has been demonstrated to induce inflammasome release from macrophages [21]. Inhibition of caspase-8 leads to the effective lower pro-inflammatory cytokine release. A study of lung tumor-bearing mice reported that inhibition of caspase-8 by z-IETD-FMK robustly reduced tumor outgrowth and this was closely associated with a reduction in the release of pro-inflammatory cytokines, IL-6, TNF-α, IL-18, IL-1α, IL-33, but not IL-1β [22].

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3.2 Interaction of metformin with caspase 3 and 7

Metformin activated the effector caspase 7, whereas has a dual action as an activator and inhibitor towards executioner caspase 3. Activation of caspase 3 or 7 was the downstream signal to execute the cell death via the apoptosis pathway. As mentioned before, activation of caspase-dependent cell death is beneficial in suppressing cancer cell progression. However, in some conditions, the occurrence of cell death is undesirable. In hyperglycemia conditions, apoptotic cell death occurs in the diabetic myocardium and has been repeatedly linked with diabetic kidney disease (DKD), which is a programmed cell death mediated by effector caspases 3, 6, and 7. The inhibition of caspase-3 with a particular inhibitor decreased apoptosis mediated by high glucose levels [23]. Therapeutic use of caspase-3 inhibition in diabetic kidney disease showed that inhibition of caspase-3 led to the nephroprotective effects by preventing the deafness gene, Gasdermin E (GSDME) activation. GSDME could switch caspase-3-dependent non-inflammatory and immunologically silent apoptosis to a terminal phase, namely secondary necrosis. Specifically, knocking down GSDME directly inhibited secondary necrosis and fibrogenesis [24].

4. Conclusion

In this bioinformatic study, metformin has been identified as capable of activating caspase-3 and -7 expressions. However, at the same time, metformin also inhibits caspase-3 and -8. It can be concluded that metformin has a dual function, namely activation and inhibition of caspase family expression.

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