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Can Mono- or Combination Therapy of Metformin with Cimetidine and Ibuprofen be a Promising Potential Therapy for Breast Cancer?

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Abstract

Metformin (MET), either alone or in combination with other drugs, has been considered a promising drug in cancer therapy. MET via activation of adenosine monophosphate-activated protein kinase (AMPK) signaling pathway and inhibiting the mammalian target of rapamycin (mTOR) mediates tumor proliferation. Moreover, as an antagonist of the histamine H2-receptor (H2R), cimetidine (CIM) is also attributed to several immune-stimulatory responses in non-immunogenic cancers. Moreover, non-efficient inflammatory responses derived from prostaglandins may contribute to interleukin-6 (IL-6) production and tumor progression, which ibuprofen (IBU) hinders. Due to the significant effects of these drugs in the modulation of antitumor immunity and hindrance of tumor progression, it seems reasonable to assess their combinational therapy in the breast cancer (BC) mouse model.

Keywords: Metformin, Cimetidine, Ibuprofen, Signal transduction, TOR serine-threonine kinases

Introduction

Combination therapy has recently been considered a cornerstone in cancer therapy due to its promising effects [1]. The burden of breast cancer increased in recent decades, so there is a need to focus more on cancer molecular biology and on the unique features and hallmarks of the tumor microenvironment [2]. For more explanation, some of the main classical hallmarks of BC are proliferative signaling pathways (estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2)), invasion and metastasis (such as EGFR), and stem cell markers (CD44, CD24, EpCAM, CD10, CD49, CD29).

Among these, cellular signaling, modulated by drugs may play a critical anticancer role via activation and promotion of antitumor immune responses [3].

Shi et al. [4] proposed that H2R blockage via CIM significantly increased tumor burden and size in a mouse model of colon cancer. In contrast, Taghipour et al. [5] indicated that combination therapy using "MET + CIM + IBU" significantly decreased tumor size and dramatically increased the survival rate in a BC mouse model. Interestingly, Shi et al. [5] have shown that activation of H2R signaling suppressed the phosphorylation of the mitogen-activated protein kinase (MAPK) signaling pathway and decreased inflammation-associated colon cancer. However, these scientists found that CIM inhibits H2R signaling, causing the activation of JNK and ERK/MAPKs, resulting in the progression of colorectal cancer [4].

Based on the study by Taghipour et al. [5], measurement of Th1 and Treg cell percentages in the spleen indicated that combination therapy of "MET + CIM + IBU" did not significantly

increase Th1 cell percentage in the spleen compared with MET alone. Likewise, a study by Pennock et al. [6] demonstrated that IBU monotherapy increased Th1 recruitment to the mammary glands in the BC model.

Due to the tumor microenvironment (TME), splenic lymphocytes may not be able to function efficiently against the tumor [5]. Therefore, evaluating the exact responder lymphocytes at the site of the tumor (tumor-infiltrating lymphocytes (TIL)) is suggested. To explain immunosuppressive TME, metabolic competition and lack of enough nutrients result in T-cell exhaustion and apoptosis even when the tumor is highly antigenic [7]. Programmed cell death protein 1 (PD-1) negatively signals to infiltrating T cells leading to decreases in glycolysis and glucose uptake in CD8+ cells. While PD-L1, via the AKT/mTOR signaling pathway, activates glycolysis and proliferation in cancer cells [8,9].

Acidic TME caused by excessive lactic acid is another barrier to the antitumor activity of effector T lymphocytes [10]. Lactate roles as a signaling molecule via a G protein-coupled receptor (GPR81), whose activation in cancer enhances the expression of PD-L1 [11,12]. Lactic acid decreases glycolysis in CD8+ lymphocytes and reduces ATP production [13]. Therefore, TME provides a toxic circumstance to evade the antitumor immune response, and as a result, primed cells are unable to stay alive in this condition [14]. Hence, studying the exact mechanism of TIL activation and function could be worthwhile.

Taghipour et al. [5] reported that BC therapy via MET alone or combination therapy inhibits Treg differentiation via down-regulation of FOXP3 and transforming growth factor-beta (TGF-β). In contrast, Kunisada et al. [15] demonstrated that MET inhibits only tumor-infiltrating Treg (Ti-Treg) but not CD4+CD25+ regulatory T cells (Treg) in peripheral lymphoid tissues. Moreover, in this research by Taghipour et al., there were no significant differences between mono and combination therapy in the percentage of splenic Treg cells.

Not only monotherapy with MET has significantly diminished FOXP3 gene expression compared with combination therapy and untreated group, but also evaluation of human CD4+CD25+ Treg cells provided solid evidence that CIM un-stabled FOXP3 via activated PI3K/AKT/mTOR signaling pathway [16]. For more explanation, a study on specimens of patients with head and neck squamous cell carcinoma revealed that treatment with MET reduced the number (41.4%) of FOXP3+ T cells at the site of the tumor [17]. Since PI3K/mTOR signaling pathway inhibition may activate regulatory T cell differentiation and function [18], it is necessary to discuss the promising effects of MET on down-regulating FOXP3+ T cells [5,17] concerning other signaling pathways such as AMPK, which affect cellular epigenetics [19]. It is possible that the combination of CIM and IBU is also antagonized by the effect of MET in inhibiting FOXP3 gene expression. Therefore, more investigations should be conducted to confirm these results.

On the other hand, the combination of MET and CIM significantly augmented T-bet gene expression compared with MET alone. Activation of PI3K/AKT/mTORC1/2 may illustrate this effect, a signal transduction axis downstream of T cell receptor (TCR) involved in T-bet expression [20]. Interestingly, when IBU used in combination with these drugs, their effects on Th1 differentiation decreased dramatically, uncovering that IBU downregulated Th1 differentiation and T-bet expression by an unknown pathway. The anti-inflammatory effect of IBU may inhibit Th1 differentiation via down-regulation of prostaglandin-endoperoxide synthase (PTGS), an enzyme involved in catalyzing the conversion of arachidonate to prostaglandin (PGH2) [21].

Nevertheless, a study on the BC model revealed that administration of IBU not only diminished tumor burden but also recruited Th1 cells to the site of the tumor, which was significantly dose-dependent: 300 -500 mg/kg [22]. Therefore, the dose of administrated drugs is also important. In the study by Taghipour et al. [5], the dosage of IBU was 30 mg/kg. On the other hand, neither T-bet gene expression nor splenic percentage of Th1 cells significantly increased in the combination of all drugs under this study. Hence it is suggested to study the monotherapy of IBU and CIM along with combination therapy, considering their appropriate dosage.

A study by Hirayama et al. [23] in a mouse model of disseminated leukemia cells has shown that MET up-regulated IFN-γ secretion of TIL, which was also confirmed by Taghipour et al. [5] via measurement of this cytokine in mouse serum. However, as a comparison, the assessment of cytokines level at the tumor site secreted by TIL is more valid than serum level.

Although all therapies in this study dramatically decreased tumor size, the difference between mono and combination therapy was not significant. Along with drug dosage and signaling, the study by Bai et al. [23] has shown that IBU monotherapy resulted in sarcoma cells' apoptosis and inhibited the cell cycle via down-regulating the PI3K/AKT/mTOR signaling pathway. Inhibition of this pathway through MET in sarcoma cells and endometrial cancer was also confirmed [24,25].

Another cytokine studied in Taghipour et al. [5] research was TGF-β which confers a decrease in its production in combination of all drugs under this study. To discuss further, MET has been shown to interact with TGF-β1 to interrupt its binding to receptors, and reduces the downstream signaling pathway of this cytokine through AMPK independent pathway, and inhibits p-Smad2/3 [26]. Moreover, activation of AMPK inhibits Smad3-mediated TGF-β production in gastric cancer [27]. However, there were fewer studies on the effects of CIM and IBU on TGF-β production, confirming that it needs to be more investigated. Moreover, when the sample for gene expression of TGF-β was collected from the tumor

site (not serum level, which shows a systemic decrease), the difference between combination therapy and MET alone was not huge. Moreover, when the sample for gene expression of TGF-β was collected from the tumor site (not serum level, which shows a systemic decrease), the difference between combination therapy and MET alone was not huge. Therefore, the assessment of biological and molecular features of cancer needs to be accurate.

In conclusion, according to the study by Taghipour et al. [5], combination therapy of MET and other anti-inflammatory drugs affects lymphocyte function and decreases tumor proliferation. Although it was reported in some parts of this article that there was no significant difference between mono and combination therapy, combination therapy could be a promising cancer treatment. In this regard, the effect of drug dose on TIL and related signaling pathways should be considered. Therefore, it is worthwhile to study the molecular mechanism of this combination therapy and highlight the signaling pathways involved in tumor proliferation and anticancer lymphocyte differentiation.

Author Contribution

S.M wrote the entire manuscript and edited it. ZM.H is the corresponding author who edited the manuscript and provided comments on it. H.Z also provided comments and edited the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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