Minireview

Similarities and Distinctions in the Effects of Metformin and Carbon Monoxide in Immunometabolism

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http://dx.doi.org/10.14348/molcells.2019.0016
www.molcells.org

Immunometabolism, defined as the interaction of metabolic pathways with the immune system, influences the pathogenesis of metabolic diseases. Metformin and carbon monoxide (CO) are two pharmacological agents known to ameliorate metabolic disorders. There are notable similarities and differences in the reported effects of metformin and CO on immunometabolism. Metformin, an anti-diabetes drug, has positive effects on metabolism and can exert anti-inflammatory and anti-cancer effects via adenosine monophosphate-activated protein kinase (AMPK)-dependent and AMPK-independent mechanisms. CO, an endogenous product of heme oxygenase-1 (HO-1), can exert anti-inflammatory and antioxidant effects at low concentration. CO can confer cytoprotection in metabolic disorders and cancer via selective activation of the protein kinase R-like endoplasmic reticulum (ER) kinase (PERK) pathway. Both metformin and CO can induce mitochondrial stress to produce a mild elevation of mitochondrial ROS (mtROS) by distinct mechanisms. Metformin inhibits complex I of the mitochondrial electron transport chain (ETC), while CO inhibits ETC complex IV. Both metformin and CO can differentially induce several protein factors, including fibroblast growth factor 21 (FGF21) and sestrin2 (SESN2), which maintain metabolic homeostasis: nuclear factor erythroid 2-related factor 2 (Nrf2), a master regulator of the antioxidant response; and REDD1, which exhibits an anticancer effect. However, metformin and CO regulate these effects via different pathways. Metformin stimulates p53- and AMPK-dependent pathways whereas CO can selectively trigger the PERK-dependent signaling pathway. Although further studies are needed to identify the mechanistic differences between metformin and CO, pharmacological application of these agents may represent useful strategies to ameliorate metabolic diseases associated with altered immunometabolism.

Keywords: heme oxygenase-1, metabolic diseases, metabolic homeostasis, mitochondrial ROS, PERK

INTRODUCTION

Cellular metabolism, which consists of the concerted actions of many thousands of genes, proteins, and metabolites, refers to the complex chemical reactions that occur in living cells. Generally, these reactions can be divided into those supporting catabolic metabolism or anabolic metabolism. Catabolic metabolism, which includes glucose transport, fatty acid β-oxidation and other processes, converts complex molecules to simpler molecules. In contrast, anabolic metabolism, which includes lipogenesis, gluconeogenesis and other...
processes, converts simple precursor molecules into more complex biomolecules such as proteins and other polymers. The reactants and products of these chemical reactions are known as metabolites. An imbalance of metabolic functions is associated with the pathogenesis of metabolic diseases (Bray, 2004; Bugianesi et al., 2010; Hanahan and Weinberg, 2011; Weyer et al., 1999). Metabolic diseases include cardiovascular disease (CVD), type 2 diabetes (DM2), stroke, chronic kidney disease (CKD) and cancer. These diseases are caused by complicated conditions such as insulin resistance, obesity, dyslipidemia, hypertension and hyperglycemia (Alberti et al., 2009; Grundy et al., 2004). The pathogenesis of metabolic disease is related to altered metabolism and immune responses, which can lead to activation and infiltration of immune cells into metabolic tissues, such as liver, adipose tissue and skeletal muscle; and chronic low-grade activation of inflammatory pathways in both stromal and immune components. These events may be followed by the activation of stress kinases, such as c-Jun N-terminal kinase (JNK); and the mammalian target of rapamycin complex 1 (mTORC1), activation of which negatively affects the signaling of metabolic hormones such as insulin, leading to dysregulated glucose and lipid homeostasis (Boucher et al., 2014; Hotamisligil, 2017; Manieri and Sabio, 2015; Wu and Ballantyne, 2017). Altered cellular metabolism is a hallmark of cancer, and contributes to the conversion to malignancy and the initiation, growth and maintenance of tumors (Hanahan and Weinberg, 2011). Metabolites in tumors can have profound effects on both cancer cells and immune system cells. For example, adenosine is known to enhance tumor progression, whereas this compound inhibits pro-inflammatory responses (Prado-Garcia and Sanchez-Garcia, 2017).

Immunometabolism refers to an emerging research field at the interface of metabolism and the innate immune system (Mathis and Shoelson, 2011). In 1966, the first observation of immunometabolism was the infiltration of macrophages into adipose tissue in obese mice (Hausberger, 1966). Specifically, the term immunometabolism describes two major processes: (I) the progression of non-immune pathologies, such as obesity, can result in the mobilization of innate and adaptive immune systems; (II) secondly, the dysbalance of internal metabolites can influence the immune response of lymphocytes and other leukocytes. Thus, metabolism and immunity have been broadly linked throughout evolution (Hotamisligil, 2017; Schertzer and Steinberg, 2014). Many studies have implicated immunometabolism during the development of metabolic disease. For example, the conditioned medium from macrophages incubated with LPS can induce resistance to insulin-induced glucose uptake and lipoprotein lipase expression in adipocytes (Pekala et al., 1983). Adipose tissue-derived TNF-α contributes to type 2 diabetes in obese mice (Uysal et al., 1997). In addition, various signaling pathways have been linked to glucose metabolism (Coppes and White, 2012; Fullerton et al., 2013). Recently, it has been reported that adipose tissue-resident immune cells play an important role in tissue remodeling during weight gain and brown adipose tissue modulation (Lee et al., 2013; Wolf et al., 2017). Therefore, the relationship between metabolic diseases and the innate immune response is closely related, such that immunometabolism can be a therapeutic target for metabolic diseases. On the other hand, the behavior of leukocytes and lymphocytes can be regulated by metabolism. Changes in CD8+ and CD4+ T cell populations have been associated with diabetes. In diabetic patients, CD8+ T cells were associated with impaired glycemic control and lipidemia in type 1 diabetes (Laban et al., 2018), and CD4+ T cells were associated with progression of subclinical inflammation in type 2 diabetes (Kumar, 2018; Sheikh et al., 2018). In addition, B cells were associated with metabolic homeostasis. Specifically, B-1a lymphocytes can attenuate insulin resistance through IL-10 and IgM-dependent mechanisms (Shen et al., 2015). Furthermore, the polarization of macrophages is important for progression of obesity (Li et al., 2018). M1 macrophages induce insulin resistance, whereas M2 macrophages protect against obesity-induced insulin resistance (Chawla et al., 2011). Metformin has been commonly used to treat DM2 (Witters, 2001). Metformin enhances the insulin response and glucose transport through adenosine monophosphate-activated protein kinase (AMPK) activation (Shaw et al., 2005). Also, metformin inhibits fatty acid synthesis, and increases β-oxidation in an AMPK-dependent manner (Zhou et al., 2001). Beyond the modulation of glucose and lipid metabolism, metformin can exert anti-inflammatory functions (Caballero et al., 2004; Dandonna et al., 2004; Kim et al., 2014). Metformin has been used to treat not only DM2 but also other metabolic diseases, such as CVD, non-alcoholic fatty liver disease (NAFLD) and cancer, in both an AMPK-dependent and –independent manner (Ben Sahra et al., 2011; Gotlieb et al., 2008; Griffin et al., 2017; Kim et al., 2013a; Zakikhani et al., 2006). Metformin, when administered at high doses (3000 mg/day) for treatment, can cause side effects such as gastrointestinal disturbances (Siavash et al., 2017) and lactic acidosis (DeFranzo et al., 2016).

Carbon monoxide (CO) is another molecule that can modulate metabolic responses. Endogenous CO is produced by the enzyme heme oxygenase-1 (HO-1) (Otterbein et al., 2016). CO has anti-inflammatory and antioxidant effects (Jamal Uddin et al., 2016; Kim et al., 2007) and can confer cytoprotection in metabolic diseases via activation of the PERK pathway (Joe et al., 2018; Kim et al., 2018b). CO-releasing molecules (CORMs), can be used as an alternative and potentially safer substitute for inhalation of gaseous CO (Motterlini et al., 2002). Furthermore, CO can have a cytoprotective effect at low concentrations (Otterbein, Foresti et al., 2016). Metformin and CO have been shown to attenuate progression of metabolic diseases, obesity, DM2 and cancer, by various molecular pathways. Therefore, further studies are needed to identify the differences between metformin and CO, and to determine if CO, which has a therapeutic effect at low concentrations, can compensate for the disadvantages of metformin, which can also incur side effects at high doses. Both metformin and CO continue to show potential for therapeutic application in metabolic diseases associated with immunometabolism, though further studies are needed.
ROLES OF METFORMIN IN IMMUNOMETABOLISM

Metformin is known as a metabolic drug that is extensively prescribed for DM2 due to its ability to enhance insulin sensitivity. Numerous studies have demonstrated that metformin regulates glucose and lipid metabolism (Cao et al., 2014; Chen et al., 2017; Gopoju et al., 2018; Zhou et al., 2016). Also, metformin has been shown to decrease various pro-inflammatory markers, including soluble intercellular adhesion molecule, vascular cell adhesion molecule 1, macrophage migration inhibitory factor and C-reactive protein (Caballero et al., 2004; Dandona et al., 2004). Metformin also influences the behavior of immune cells in response to metabolic mediators. For example, metformin can enhance B cell responses through a reduction in B cell-intrinsic inflammation in individuals with obesity and DM2 (Diaz et al., 2017). Metformin was also shown to regulate the immune response by alteration of macrophage polarization and T cell infiltration in a zebrafish model of NAFLD-associated hepatocellular carcinoma (de Oliveira et al., 2019). Furthermore, it has been reported that metformin can exert anti-inflammatory effects, which are related to an alteration in macrophage polarization to the M2 phenotype through activation of AMPK in a HFD-induced model of obesity, and in palmitate-stimulated macrophage in vitro (Jing et al., 2018). The intracellular target of metformin is the mitochondria, where metformin transiently inhibits complex I of the mitochondrial ETC, which results in a decline in energy charge. This inhibition of complex I induces a mild elevation in mitochondrial reactive oxygen species (mtROS) (Kim et al., 2013a), a decrease in ATP production and an increase in AMP levels which drive the activation of AMPK (Zhou et al., 2001)(Fig. 1).

AMPK acts as an energy and nutrient sensor and coordinates an integrated signaling network that constitutes metabolic and growth pathways. Metformin-induced AMPK activation exhibits enhancement of glucose transport (Gulton et al., 2003) and inhibits gluconeogenic gene expression via the cAMP-response element-binding protein (CREB) and the CREB-regulated transcription coactivator 2 (CRTC2) (Lee et al., 2010)(Fig. 1). AMPK increases the activity of the insulin receptor and insulin receptor substrate (IRS) by phosphorylation of these molecules, and then enhances the insulin response and glucose transport (Grisouard et al., 2010)(Fig. 1). AMPK also inhibits fatty acid synthesis by reducing lipogenic gene expression through transcription factors such as sterol regulatory element binding protein-1c (SREBP-1c) carbohydrate-responsive element-binding protein (ChREBP); and enhances β-oxidation by regulating multiple enzymes involved in β-oxidation (Xu et al., 2013; Zhou et al., 2001) (Fig. 1). Metformin has positive effects on metabolism beyond glucose metabolism and also suppresses pro-inflammatory markers (Kim et al., 2018a). Metformin has been reported to suppress lipopolysaccharide (LPS)-stimulated inflammation via AMPK-activating transcription factor 3 (ATF-3) (Kim et al., 2014). Metformin has also been reported to reduce inflammation through p53-coordinated sestrin2 (SESN2)-AMPK-mTOR signaling in mouse embryonic fibroblasts (Deng et al., 2016)(Fig. 2). SESN2 is a target of the tumor suppressor gene p53, and negatively regulates mTOR signaling. Several studies have demonstrated that metformin activates nuclear factor erythroid 2-related factor 2 (Nrf2) in an AMPK-dependent manner, and thereby exerts antioxidant and anti-inflammatory effects under conditions of global cerebral ischemia (Ashabi et al., 2015)(Fig. 2). Metformin can extend lifespan through activation of the liver
kinase B (LKB)-AMPK-Nrf2 pathway in *C. elegans* (Onken and Driscoll, 2010). Thus, metformin prevents metabolic diseases through AMPK-induced cellular pathways. Metformin has been known to prevent metabolic diseases including diabetes and obesity, and also cancer, through AMPK activation. mTOR induces energy-consuming cellular responses and controls cell growth. Activation of AMPK inhibits the mTOR pathway by phosphorylation of tuberous sclerosis complex (TSC), a tumor suppressor gene (Howell et al., 2017)(Fig. 2). AMPK-dependent inhibition of mTOR induces anti-proliferation and inhibits cell viability in several types of cancer (Gotlieb et al., 2008; Zakikhani et al., 2006). Therefore, metformin also protects cancer cell proliferation and growth via the AMPK-mTOR pathway.

Several findings support the notion of an AMPK-independent pathway in metabolic diseases. In this context, AMPK-independent effects of metformin have been described. Metformin has been shown to inhibit hepatic gluconeogenesis, which is mediated in an AMPK-independent manner via a decrease in hepatic energy state (Foretz et al., 2010). In addition, metformin provokes mild mtROS production through inhibition of complex I, and then induces fibroblast growth factor 21 (FGF21) through a mitochondrial stress-induced unfolded protein response (UPR) pathway, known as the endoplasmic reticulum (ER) stress-sensing pathway (Kim et al., 2013a)(Fig. 2). ROS have been reported to induce the PERK/ eukaryotic initiation factor-2 alpha (eIF2α) activation transcription factor-4 (ATF4) pathway, which is one of the three branches of the UPR (Liu et al., 2008). Also, it has been reported that metformin activates the PERK-eIF2α-ATF4 pathway but not the other branches of the UPR represented by activating transcription factor 6 (ATF6) and inositol-requiring transmembrane kinase/endonuclease 1α (IRE1α)(Quentin et al., 2012). ATF4 prevents obesity and insulin resistance by upregulation of FGF21, an endocrine hormone that exerts effects of anti-obesity and anti-diabetes (Kim et al., 2013b). In cancer treatment, metformin exerts anti-cancer effects via an AMPK-independent pathway. Metformin, independent of AMPK, exhibits an anticancer effect through the p53-REDD1 (regulated in development and DNA damage responses-1) pathway in a prostate cancer cell line (Ben Sahra et al., 2011)(Fig. 2). REDD1, known as RTP801, Dig2 and DDIT4, was identified as a hypoxia-inducible factor (HIF)-1 target gene associated with the regulation of cell survival, and negatively regulates mTOR (Brugarolas et al., 2004; Shoshani et al., 2002). Therefore, REDD1 exhibits an anticancer effect, via inhibition of cell growth and cell cycle arrest, through mTOR inhibition. Furthermore, metformin has been shown to inhibit mTORC1, which is mediated in an AMPK-independent manner via Rag GTPase inhibition (Kalender et al., 2010)(Fig. 2). In summary, the ability of metformin to prevent immune-metabolic diseases can be explained by both AMPK-dependent and AMPK-independent mechanisms.

**ROLES OF CARBON MONOXIDE IN IMMUNOMETABOLISM**

Cells and tissues have been known to induce an adaptive response to stress (Otterbein et al., 2003), which is responsible for defending against damage and preserving cellular homeostasis, and depends on the induction of several beneficial defense systems (Otterbein et al., 2016). Among these, the stress protein HO-1 can defend against cellular damage by the catalysis of heme, a pro-oxidant molecule (Otterbein et al., 2016). HO-1 can be induced by numerous agents, including...
CO, curcumin, resveratrol and flavonoids (Chen et al., 2005; McNally et al., 2007; Szabo et al., 2004; Yang et al., 2014) (Fig. 3). The major function of HO-1 is to degrade heme which results in the generation of CO and biliverdin, and the release of iron (Otterbein et al., 2003). Many reports suggest that the reaction products of HO-1 (i.e., CO, biliverdin and iron) acting individually or in concert, can mediate its cytoprotective effects. In this section, the positive effects of CO are described, with respect to immune responses and metabolism.

CO can exert cytoprotective and therapeutic effects in several disease models (Otterbein et al., 1999; 2000; Motterlini et al., 2002; Morse et al., 2003). Also, CO can have a cytoprotective effect at low concentrations (Otterbein et al., 2002), but may be impractical for clinical use in the gaseous state (Smalilova et al., 2018). CORMs may be used to exert similar effects as inhalation of gaseous CO in a controlled manner (Motterlini et al., 2002). CORM compounds, typically transition metal carbonyls that can release CO, include CORM-1, CORM-2, CORM-3 and CORM-A1 (Foresti et al., 2004; Motterlini et al., 2002; 2005) (Fig. 3). CORM-1 [Mn2(CO)10] was the first of such compounds developed, and is restricted as a pharmacological agent because it is insoluble in aqueous media and requires photoactivation to release CO (Motterlini et al., 2002). CORM-2 [Ru(CO3Cl)2] is hydrophobic and can release CO from organic solvents (Motterlini et al., 2002). Further studies have identified water-soluble CORM-3 [Ru(CO)3Cl(glycinate)] and CORM-A1 [Na2H3BCO2]. These CORMs are known to release CO in aqueous media (Foresti et al., 2004; Motterlini et al., 2005). Therefore, these CORMs can potentially treat many disease models by cytoprotective action including antioxidation action. CO has been shown to suppresses the pro-inflammatory response, which is mediated by decreasing the production of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), in the presence of LPS; and by promoting the production of the anti-inflammatory cytokine IL-10, both of which are mediated by activation of the p38 mitogen-activated protein kinase (MAPK) (Otterbein et al., 2000). Furthermore, CO can generate itaconate, which exerts immunosuppression and anti-microbial functions via induction of immune-regulator genes then inhibit pro-inflammatory responses (Jamal Uddin et al., 2016). CO has been shown to be protective in models of diet-induced obesity, insulin resistance, NAFLD and cancer (Hosick et al., 2014; Joe et al., 2018; Kim et al., 2017; 2018b). CO and CORM-2 were shown to suppress T cell proliferation through IL-2 production (Pae et al., 2004). CO derived from CORM-A1 inhibited T helper 17 differentiation, which is related to the immunomodulatory effect of CO (Takagi et al., 2018). In macrophage polarization, CORM-3 attenuated the expression of iNOS, a marker of M1-polarized macrophage; and upregulated the expression of CD206 and Ym-1 protein, markers of M2-polarized macrophages, in LPS and IL-4-stimulated alveolar macrophages, respectively (Yamamoto-Oka et al., 2018). CO induces low levels of mtROS through blocking of mitochondrial ETC complex IV, and subsequently increased mtROS can activate the PERK/eIF2α/ATF4 signaling pathway (Liu et al., 2008; Otterbein et al., 2016; Zuckerbraun et al., 2007)(Fig. 3). It also has been reported that CO can activate Nrf2, the master regulator of the antioxidant response, via PERK activation (Caballero et al., 2004)(Fig. 3). ATF4 upregulates several genes, FGF21, SESN2 and REDD1, through PERK pathway activation (Kim et al., 2013b; 2017; 2018b; Joe et al., 2018)(Fig. 4). Under high fat diet conditions, CO can increase FGF21 expression by activating the PERK pathway in liver, resulting in metabolic homeostasis through lipolysis of white adipose tissues, conversion from white adipocyte to beige adipocyte, and alleviation of fatty liver (Joe et al., 2018)(Fig. 4). Under methionine-choline deficient (MCD) diet conditions, CO has been shown to increase the expression of SESN2 through PERK activation, which activates AMPK which in turn inhibits mTOR signaling to induce autophagy, resulting in the amelioration of MCD-induced steatohepatitis (Kim et al., 2017)(Fig. 4). CO induces REDD1 through PERK activation, and subsequently, REDD1 can inhibit cell cycle arrest, growth and metastasis of tumors by inducing mTOR inactivation, and apoptosis (Kim et al., 2018b)(Fig. 4). Therefore, CO has been shown to prevent diet-induced obesity, insulin resistance and NAFLD via FGF21 and SESN2; and to prevent tumor growth and metastasis via REDD1. Thus, CO-induced protective effects in metabolic diseases can occur in a PERK-dependent manner.

SIMILARITIES OF METFORMIN AND CARBON MONOXIDE

Metformin and CO generate ROS through blocking of mitochondrial ETC. The generated-ROS activate PERK/eIF2α/ATF4 signaling, one of the branches of the UPR, but not IRE1α and ATF6. Metformin and CO both increase the expression levels of FGF21, SESN2 and REDD1, and also activate Nrf2. Specifically, FGF21 was induced by metformin and CO via PERK-activated ATF4. Therefore, metformin and CO exert positive effects in metabolic diseases, such as obesity, insulin...
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Fig. 4. Carbon monoxide controls metabolic homeostasis via the PERK-eIF2α-ATF4-FGF21, SENS2, and REDD1 pathway. See the text for more details.

Table 1. Similarities and distinctions of metformin and carbon monoxide

| Similarity | Metformin | Carbon monoxide |
|------------|-----------|----------------|
| - Exhibiting effects of cytoprotection by production of ROS in mitochondria (Kim et al., 2013a; Liu et al., 2008; Zuckerbraun et al., 2007) | | |
| - Activation of the PERK pathway by induction of mitochondrial stress (Joe et al., 2018; Kim et al., 2013a; 2017; Quentin et al., 2012) | | |
| - Induction of metabolic homeostasis molecules (e.g., FGF21 and SESN2) and anti-tumor and anti-oxidant molecules (e.g., REDD1 and Nrf2, respectively) (Ben Sahra et al., 2011; Deng et al., 2016; Kim et al., 2007; 2013a; 2018b) | | |
| Distinction | Metformin | Carbon monoxide |
| - Increase of ROS by partial inhibition of mitochondrial complex I (Kim, Jeong et al., 2013a) | - Production of ROS via temporarily blocking cytochrome c oxidase, complex IV (Otterbein et al., 2016; Zuckerbraun et al., 2007) |
| - Induction of SESN2 and REDD1 by the p53-dependent pathway in mouse embryonic fibroblasts and prostate cancer cell lines, respectively (Ben Sahra et al., 2011; Deng et al., 2016) | - Induction of SESN2 and REDD1 by PERK-dependent pathway in liver and several cancer cell lines, respectively (Kim et al., 2017; 2018b) |
| - Activation of Nrf2 in an AMPK-dependent manner in rat brain and C. elegans (Ashabi et al., 2015; Onken and Driscoll, 2010) | - Activation of Nrf2 via PERK activation in human endothelial cells (Kim et al., 2007) |

resistance, NAFLD and cancer, through the regulation of the metabolic homeostasis molecules, FGF21, SESN2, REDD1 and Nrf2 (Table 1).

DISTINCTIONS OF METFORMIN AND CARBON MONOXIDE

Metformin induces ROS via inhibition of mitochondria ETC complex I. The elevation in ROS increases the level of AMP, leading to activation of AMPK. The increased AMPK activation enhances insulin sensitivity through improved glucose transport and activity of the insulin receptor and IRS, and regulates fatty acid synthesis and β-oxidation, and subse-
CO upregulates REDD1 via PERK-dependent ATF4 which transcriptional regulation of REDD1 through p53. In contrast, Metformin inhibits proliferation of cancer cells through cation by PERK activation. Nrf2 in turn upregulates HO-1 pathways and inhibits inflammatory responses through induction of AMPK after transient global cerebral ischemia. Metab. Brain Dis. 30, 298  Mol. Cells 2019; 42(4): 292-300

ACKNOWLEDGMENTS
This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2014R1A6A1030318) to H.T. C.

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