Chapter

Metformin: A Small Molecule with Multi-Targets and Diverse Therapeutic Applications

Farid A. Badria, Ahmed R. Ali, Ahmed Elbermawi, Yhiya Amen and Adel F. Badria

Abstract

Metformin is one of the most prescribed agents in the treatment of type 2 diabetes. Its history goes back to the use of goat's rue (*Galega officinalis* Linn., Fabaceae). *G. officinalis* is rich in galegine, a guanidine derivative with a blood glucose-lowering effect. Research based on the effects of guanidine rich on this traditional herbal medicine led to the development of metformin. Metformin continues to serve as a multi-target drug. Its benefits for treating/controlling several diseases were thoroughly discovered over time. These include health disorders such as cancers, obesity, periodontitis, cardiovascular, liver, skin, and renal disorders. Moreover, there is evidence to propose that metformin postpones the aging processes as well as modulates the microbiota to promote better health. So far, it is not fully understood, how metformin can accomplish such pleiotropic pharmacological and therapeutic effects. Metformin may decrease malignancy via suppressing the signal of insulin/IGF-1, avoiding the release of cytokines via NF-κB, and increasing the immune reaction to cancer cells. This chapter discusses the history of metformin discovery, chemistry, its role in diabetic patients, and proposed molecular mechanisms to shed more light on the diverse effects and its ability to target multiple signaling pathways.

Keywords: metformin, galegine, biguanides, type 2 diabetes, drug repurposing, cancer, metabolic disorders, degenerative diseases

1. Introduction

Currently, polyfunctional or polypharmacy or multiple targets may present new therapeutic avenues for drugs for the treatment of many chronic and/or complex health disorders which constitute multifactorial pathogenesis, for example, genetics, environment, and lifestyle.

Recently, poly-target molecules including metformin have been recognized and efficiently used in the treatment of several metabolic disorders, infectious, and inflammatory diseases.

2. Concept of multi-target drugs

Polyfunction or polypharmacy or multiple targets have emerged as new potential therapeutic drugs for the treatment of chronic and/or complex diseases. It is well-known that many chronic diseases are usually polyfactorial including different genetics, environment, constitutive, and mixed aspects [1].

Recently, poly-target molecules have been recognized and efficiently used in the treatment of several metabolic disorders, infectious, and inflammatory diseases [1] Promiscuous enzyme is an enzyme that is capable to catalyze several reactions; for example, lipase enzyme is capable to convert lipids or phospholipids or triglycerides to corresponding fatty acids and alcohols [2]. Also, the promiscuity of substrate (aryl esterase of carboxylic acids) acts on amide bond and could be called catalytic promiscuity. Moreover, there is another class of promiscuity based on the capability of small molecules (e.g., valproate, aspirin, and quinine) to selectively react with many receptors leading to diverse pharmacological activities [2].

Acetylsalicylic acid proved to act by different physiological and pharmacological targets; for example, cyclooxygenase enzyme which may modulate several signaling pathways, and subsequently prompt poly-target-dependent pharmacological activities [1].

3. Approaches for developing multi-targets agents

3.1 Approach 1 (fragment-based)

Choosing a combination of different pharmacophores from single-target ligands and screening a series of compounds using several computational models and/or via optimizing in vitro assays [3].

3.2 Approach 2 (selection-based target)

Based upon the selection of preferentially expressed protein/antigen as manifested in many infectious diseases or cancer. These selected proteins must not retain homologous proteins in the same person or the selected proteins ought to be different to keep the selectivity [1, 3]. However, in this approach many other parameters need to be considered:

1. The characteristics of the disease under investigation (inflammatory, infectious, metabolic, or complex).

2. The mode of drug/compound resistance (adaptive or mutation or amplification of target amplification) [1].

3.3 Approach 3 (molecular docking or computer-assisted drug [CAD])

The design of CAD may be used to examine the possible ligand (drug) interaction with a receptor (target) to figure out the existing energy and possible extent of interaction. Therefore, based on this approach, we may arrange the existent ligand–target interactions based on the calculated energy. The prediction of interaction will be based on binding energy between a drug/compound and a target [4].

4. Applications of multi-targets compounds

4.1 Complex health disorders (CHDs)

Several inherent and/or environmental factors represent the major causes of the complex health disorders as presented in **Figure 1**. There are many examples of CHDs but not limited to malignancy.

4.2 Drug resistance

Drug resistance may be defined as a decrease in the efficacy of certain drugs; for example, anticancer, antimicrobial, and anti-epilepsy in curing or alleviating the symptoms and/or conditions of a certain disease. Moreover, the term "drug resistance" could be used alternatively in acquiring resistance to many types of cancers or common pathogens.

Generally, drug resistance may be attributed to one or more of the following mechanisms [1–4]:

- Releasing of drug/compound-inactivating enzymes
- Alteration of an existing receptor
- Acquisition of a site/target by-pass system
- Reduction of permeability of cells

The activity of metformin on modulating drug resistance of several types of cancers may influence the proliferation and metastasis of resistant cells resistant to

Figure 1.
Complex health disorders (CHDs) may present complex and combined factors; for example, genetics, environment, and lifestyle which may represent the major causes of complex health disorders, metabolic disorders, chronic degenerative, inflammatory, and neurological disorders. Multi-target compounds are very much valuable in the modulation of CHDs where patients may avoid the risk of drug–drug interaction, drug intolerance, and sustain better tolerance and pharmacokinetics [4].

tamoxifen or paclitaxel in the case of breast cancer, for example, via focusing on many changes in the Scribble (SCRIB)-induced activation of the Hippo pathway [5].

Metformin (MET), which exhibits anti-cancer activity via induction of AMP-activated protein kinase (AMPK), directly phosphorylates YAP and inhibits YAP transcriptional activity [6, 7].

4.3 Drug repositioning

Drug discovery or development of new drugs can be very lasting, costly, prone to many side effects, tolerability, and toxicity, as well as high cost [8]. Therefore, drug repositioning or drug repurposing may consider a well-accepted concept. For the last decade, metformin has been extensively proven high safety and tolerability in many clinical trials, especially when used as adjuvant therapy in many resistant drug cancer [8].

Drug repurposing may include a broad spectrum of old or recently approved drugs; for example, FDA-approved drugs or old, or even shelved drugs. Serendipity was the main avenue for discovering new uses for commonly available old drugs. However, computer-assisted drugs (CAD) showed to be an efficient method for retrospectively discovering new uses/targets for several known drugs [9].

5. Metformin: a unique model for small molecules with multi-target effects

Metformin molecules retained unique chemical and dynamic features. Chemically, metformin is a derivative of guanidine, one of the closely related active components in *Galega officinalis* Linn. (goat's rue) [9]. Chemical studies of *G. officinalis* showed that the plant was rich in guanidine and related compounds and proved to have hypoglycemic activity in animals but retained high toxicity in humans [10]. Therefore, a less toxic component of *G. officinalis*, galegine (isoamylguanidine), has been brought

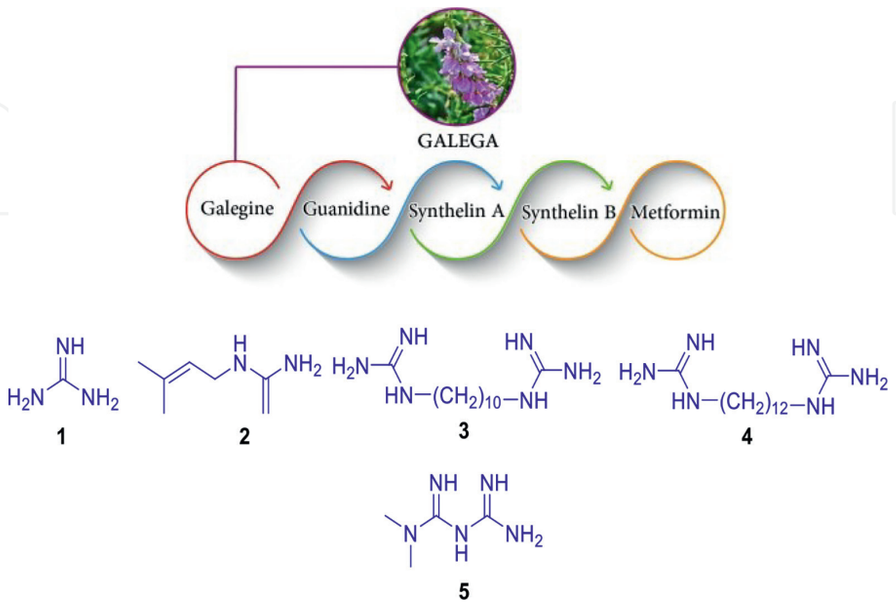

Figure 2.
Evolution of metformin discovery from Galega officinalis plant; guanidine (1), galegine (2), synthelin A (3), synthelin B (4), and metformin (5).

DOI: http://dx.doi.org/10.5772/intechopen.108884

to light and it was used as an antidiabetic agent in the 1920s. Unfortunately, its toxicity caused it to be swiftly discontinued. However, the unique chemical nature of galegine has led the pharmaceutical industry to a new drug lead with lipophilic nature [11]; for example, Synthelin A (decamethylene diguanide) and Synthelin B (dodecamethylene diguanide) with better activity, tolerability, and unique dynamic and energetic structure as well as the interaction features with DNA minor grooves with AT-rich minor grooves of DNA [11–14] (**Figure 2**).

Generally, metformin, a biguanide derivate, is considered a safe drug. However, the evolution for developing metformin starts from galagine (1) which was isolated from *G. officinalis* plant, then followed by guanidine (2), biguanidine with (-CH2-)10 chain, (-CH2-)12 chains as a linker between the two guanidine moieties for Synthalin A (3) and Synthalin B (4), respectively. Unfortunately, even though their antihyperglycemic activity was better than metformin but showed to be more toxic with several side effects.

6. Metformin uses in CHDs

There are diverse and multiple therapeutic targets; inflammation, metabolic disorders, cancer, and degenerative diseases which are revealed to be hit by metformin as presented in **Figure 3**.

6.1 Metabolic disorders

This may include type 2 diabetes (DM-Type II), obesity, atherosclerosis (CVDs, NASH), inflammation, and infectious disorders.

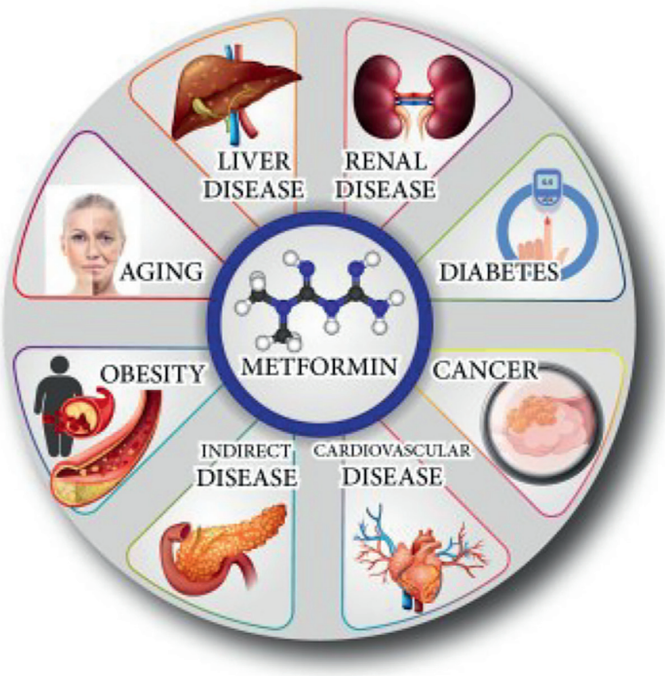

Figure 3.
Metformin has been recognized and efficiently used in the treatment of several metabolic disorders, infectious, and inflammatory diseases. Having an enzyme capable to catalyze several reactions (Promiscuous enzyme); for example, lipase enzyme is capable to convert lipids or phospholipids or triglycerides to corresponding fatty acids and alcohols.

6.1.1 Hepatic gluconeogenesis

Metformin can regulate hepatic gluconeogenesis via different mechanisms; for example, activation of AMPK which leads to accumulation of AMP and subsequently reduces the level of cAMP via inhibition of adenylate cyclase. Moreover, glycerophosphate dehydrogenase (mGPD) in mitochondria (enzyme responsible for converting glycerol to dihydroxyacetone phosphate). This inhibitory activity of mGPD will lead to the augmentation of both glycerol and glycerol-3-phosphate and suppress gluconeogenesis via alteration of the redox state in the cytosol [15].

6.1.2 Gut microbiome and inflammation

The gastrointestinal (GIT) physiology with its gut microbiota in glucose metabolism plays a major role in metformin efficacy and tolerance. Healthy GIT may contain huge numbers of microorganisms not limited to bacteria and fungi but may have viruses, protozoa, and fungi. The collective microbiome genomes encode over 100-fold genes more than the human genome generating many metabolites which can affect the health of the human; for example, inflammation, immunity, and microenvironment metabolism as production of fatty acid (short-chain fatty acid [SCFA]) which may lead to type 2 diabetes [16]. Therefore, a clinical study proved the capability of metformin to increase the number of beneficial bacteria such as *E. coli* species which proved to reduce the risk of the incidence of type 2 diabetes. On the other hand, the role of the microbiome as a cause or result for therapeutic benefit would require further investigation [17].

Metformin multi-targeted actions are induced through non-AMPK pathways and AMPK mechanisms [18]. Therefore, a wide range of beneficial effects were observed in several systems of the body including GIT, lung, kidney, pancreas, and CVS [18].

6.1.3 Cardiovascular systems

A prospective study carried out in the UK (UKPDS) showed that cardiovascular diseases are a major complication of type 2 diabetes [19]. Separately, in the USA the program for the prevention of type 2 diabetes revealed the significant role of metformin in the prevention of type 2 diabetes via its effects on impaired glucose tolerance (IGT) [20].

Metformin has shown positive progress in the number of clinical outcomes of CVD, including dyslipidemia, endothelial dysfunction, and systemic inflammation [21]. Regarding endothelial dysfunction, Metformin does this improvement by increasing nitric oxide synthase (eNOS) releasing the vasodilator agent, nitric oxide (NO). Additional pathways include stimulation of AMPK, inhibition of mitochondrial complex 1, and suppression of apoptosis [22].

Metformin has shown favorable effects on blood flow in several studies. It was able to enhance the reaction of hemodynamics to amino acid L-arginine (early NO precursor, vasodilator agent) [23]. Metformin also decreased dimethylarginine levels (NOS endogenous inhibitor) and reduce clot formation via diminishing platelet activity reduced through extracellular mitochondrial DNA (mtDNA) release [24].

6.1.4 Inflammation and infection

Metformin was initially used in many infectious diseases; for example, influenza and as a synergetic drug treatment in broad numbers of common diseases [25].

DOI: http://dx.doi.org/10.5772/intechopen.108884

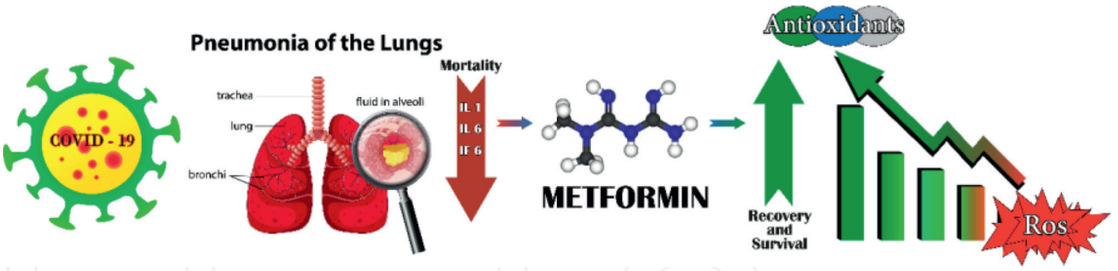

Figure 4.
Metformin with unique chemical features, multiple therapeutic targets, high safety therapeutic margin, and affordable economic cost may contribute to reducing mortality and improving recovery due to pandemic diseases; for example, COVID-19 and its fatal complications.

Repurposing metformin for fighting the current pandemic of COVID-19 represented a promising strategy as an adjunctive therapy by altering the immune responses to treat infections [26, 27]. A great advantage of metformin use is its lower mortality rate in patients with type 2 diabetes affected by COVID-19 when compared to its counterparts, especially among women population with obesity [28, 29]. Patients with COVID-19 infection who received metformin developed lower levels of interleukins [30] and other inflammatory bio-markers compared to non-users [31]. In addition, patients with type 2 diabetes are likely to develop upper respiratory diseases as well as their complications as presented in **Figure 4**; for example, chronic obstructive pulmonary diseases [32].

Long-term treatment with metformin among patients with type 2 diabetes was linked with a decreased ratio of neutrophil/lymphocyte, compared with sulfonylurea antidiabetic drugs [33]. In addition, metformin showed a protective effect among patients with type 2 diabetes regarding pneumonia-related hospitalizations and mortality rates [34].

In a clinical trial comparing metformin with placebo, the study revealed that metformin decreases the extent of pneumonia and also all pro-inflammatory cytokines among treated patients when compared with the placebo group [35]. The metformin protective effects against pulmonary infections have been hypothesized according to several mechanisms. In lung injury induced by hypoxia in animal models, metformin reduced the level of inflammatory mediators; for example, cytokines IL-6 and TNF-alpha [36]. Such traps, when overexpressed may result in exaggerated inflammatory responses with damaging effects [37].

6.2 Breast cancer

Insulin resistance could frequently contribute to the co-existence of type 2 diabetes, obesity, and cancer. This insulin resistance would result in abnormal cell growth via over-stimulation of the insulin/insulin-like growth factor pathway [38]. Other epidemiological studies showed that glycemic variability adds also some burden to cancer-related death in type 2 diabetes [39]. In the case of breast cancer, metformin exerted anticancer effects by changing the metabolic environment. This is achieved via reducing the levels of circulating insulin by improving insulin resistance related to phosphoinositide 3-kinases (PI3K) signaling [40]. Activating AMPK and liver kinase B1 (LKB1) with metformin via inhibition mTOR pathway in cancer cells. This would ultimately reduce protein synthesis and cell growth [41]. In addition, the signal transducer and activator of transcription 3 (STAT3) play a contributing role through

the AMPK and LKB1 pathway which activated apoptosis in triple-negative breast cancer. Metformin had also been shown to shift the balance of sphingosine phosphate toward ceramides which will result in the inhibition of cell growth. Additional anti-cancer actions of metformin involved increased oxidation of fatty acids and reduced transcription factors expression implicated in cancer proliferation [40].

Metformin-induced programmed cell death of cancer cells in experimental studies with inhibition of vascularization of tumor cells through vascular endothelial growth factor A. In addition, metformin proved its ability to increase patients' responses to programmed death-ligand 1 (PD-L1) chemotherapy via decreasing glucose and consumption by tumor cells [37]. Metformin also helps to overcome the chemoresistance of endometrial cancer cells by changing the epigenetic signature of tumor cells [38]. Metformin showed the ability to reduce the incidence of cancers as shown in several observational studies [39, 40]. A cohort study on patients in the UK showed that metformin monotherapy was associated with a decreased cancer risk, compared with antidiabetic sulfonylureas.

Activating the JNK/p38 MAPK pathway via metformin was proved to be contributing mechanism by metformin to induce an apoptosis-mediated effect. This would lead to growth inhibition and induce the expression of DNA damage-inducible gene 153 (GADD153) [41]. Tseng et al. provided data proposing that metformin could reduce MAPK-mediated paclitaxel-induced expression of excision repair cross complementary 1 [42]. Human epidermal growth factor receptor-2 (HER2) is a member of the epidermal growth factor receptor family. HER2 is overexpressed in nearly 20–30% of breast cancers. In human breast cancer cells, AMPK-independent inhibition of mTOR was induced by metformin which suppressed HER2 oncoprotein overexpression [43]. In addition, combination therapy of metformin with trastuzumab, anti-HER2 monoclonal antibody, could eliminate cancer stem cell populations in HER2-positive breast carcinoma cells [44].

Cancer stem cells, or tumor-initiating cells, are a group of cancer cells that have unlimited ability to regenerate resulting in tumorigenesis [45]. Cancer stem cells are believed to be both chemoresistant [46, 47] and radioresistant [48, 49]. They may be responsible for cancer metastasis and relapse, which are the major obstacle to increasing the overall survival of cancer patients. In 2009, the inhibition of cancer stem cells by metformin was first proven clinically among breast cancer models [50]. Subsequently, metformin was used as a chemo-sensitizers in cancer xenografts to eradicate cancer stem cells in multiple cancer types [51]. Metformin was able to suppress cell proliferation and migration in pancreatic cancer by deregulating miRNAs of cancer stem cells [52]. In addition, metformin inhibits esophageal and made cells more sensitive cells to 5-fluorouracil cytotoxic effects leading to the inhibition of cancer cell growth [53]. Song et al. reported that the mechanism by which metformin targets cancer stem cells is via increasing the sensitivity to radiotherapy. This would activate AMPK and suppress mTOR and help to overcome radioresistance of cancer stem cells [54].

6.3 Degenerative and cognitive disorders

Type 2 diabetes frequently coexists with aging and cognitive dysfunction. Alzheimer's disease (AD) involves the formation of beta-amyloid plaques, neuroinflammation, and neuronal loss which causes dementia in patients with or without type 2 diabetes. Metformin demonstrated some capabilities in some experimental studies to prevent amyloid plaque formation via AMPK-dependent mechanisms [55]. Metformin improved neuro-glial cell differentiation and survival at the microenvironment level

partially due to its anti-inflammatory effects [56]. Additionally, metformin might facilitate infarction-induced neural tissue repair which is done via favoring an M2 phenotype [57]. In animal studies, metformin-treated rats demonstrated a reversal of cognitive impairment caused by scopolamine [58]. In a rat model of forebrain ischemia, metformin treatment over 7 days in a rat model of forebrain ischemia resulted in the restoration of regulation of the AMPK-derived neurotrophic factor/protein S6 kinase mechanism with enhanced learning and memory [59]. One of the main confounders of the effects of metformin on cognitive function is its inhibitory effect on vitamin B12 intestinal absorption [60, 61]. In a pilot study including nondiabetic adults, a daily dose of 2 g metformin for a treatment time of 8 weeks was associated with improvement in executive function and other measures related to learning, attention capacity, and memory [62]. Taken together, these results suggested that metformin showed favorable effects on cognitive function depending on the duration and dose of metformin treatment.

7. Conclusions

In conclusion, metformin has been recognized and efficiently used in the treatment of several metabolic disorders, infectious, and inflammatory diseases [63]. Having an enzyme capable to catalyze several reactions (Promiscuous enzyme); for example, lipase enzyme which is capable to convert lipids or phospholipids or triglycerides to corresponding fatty acids and alcohols.

Moreover, having a drug with a unique chemical feature, multiple therapeutic targets, high safety therapeutic margin, and affordable economic cost [64, 65] may contribute to reducing mortality and improving recovery due to pandemic diseases; for example, COVID-19 and its fatal complications.

8. Future perspective

Nowadays, degenerative diseases, cancer, autism, psychological disorders, and many other genetic diseases may be targeted by the newly developed polyfunctional or mult-target drugs.

Recently, poly-targets molecules including metformin have been recognized and efficiently used in the treatment of several metabolic disorders, infectious, and inflammatory diseases. The capability of small molecules or drugs to react in a selective manner with different receptors to produce broad pharmacological effects may pose another useful class of promiscuity.

We do encourage all researchers to work vividly to develop as many multi-drugs as we can either via choosing a combination of different pharmacophores from single-target ligands or via the selection of preferentially expressed protein/antigen as manifested in many infectious diseases or cancer or via examining the possible ligand (drug) interact with a receptor (target) to figure out the existing energy and possible extent of interaction.

Acknowledgements

The authors would like to acknowledge the great help and support of STDF, for the continuous support and financial fund for STDF grant within the

framework of the German Egyptian Research Fund (GERF), Project ID: 33601, "A novel innovative Approach for the Optimization of Phyto-pharmaceuticals: Modulation of Medicinal plants Constituents by Elicitation", Principal Investigator: Farid A. Badria. Also, the authors would like to acknowledge the contribution of Mr. Mohamed G. Farrag, Faculty of Pharmacy, Mansoura University, Mansoura 35516 Egypt for his valuable graphic work.

Conflict of interest

No conflict of interest.

Abbreviations

AD	Alzheimer's disease
AMPK	Adenosine monophosphate-activated protein kinase
CAD	Computer-assisted drug
cAMP	Cyclic adenosine monophosphate
CHDs	Complex health disorders
COVID-19	Coronavirus disease of 2019
CVS	Cardiovascular system
eNOS	Endothelial nitric oxide synthase also referred to as NOS3 or NOSIII
FDA	Food and Drugs Administration
GADD153	Growth arrest- and DNA damage-inducible gene 153
GIT	Gastrointestinal tract

Metformin: A Small Molecule with Multi-Targets and Diverse Therapeutic Applications
DOI: http://dx.doi.org/10.5772/intechopen.108884

Author details

Farid A. Badria[1*], Ahmed R. Ali[2], Ahmed Elbermawi[1], Yhiya Amen[1] and Adel F. Badria[3]

1 Faculty of Pharmacy, Departments of Pharmacognosy, Mansoura University, Mansoura, Egypt

2 Medicinal Chemistry, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

3 Department of Fibre and Polymer Technology, KTH Royal Institute of Technology, Stockholm, Sweden

*Address all correspondence to: badri002@mans.edu.eg

References

[1] Badria FA, Ahmed MH. Multi-targets drugs: A new therapeutic approach. International Journal of Research in Pharmaceutical and Nano Sciences. 2018;**7**(5):205-213

[2] Badria FA. Drug Repurposing – Hypothesis, Molecular Aspects and Therapeutic Applications. London, UK, Rijeka: IntechOpen; 2020

[3] Koeberle A, Werz O. Multi-target approach for natural products in inflammation. Drug Discovery Today. 2014;**19**(12):1871-1882. DOI: 10.1016/j.drudis.2014.08.006

[4] Xie L, Xie L, Kinnings SL, Bourne PE. Novel computational approaches to polypharmacology as a means to define responses to individual drugs. Annual Review of Pharmacology and Toxicology. 2012;**52**(1):361-379. DOI: 10.1146/annurev-pharmtox-010611-134630

[5] Kell D. Finding novel pharmaceuticals in the systems biology era using multipleeffective drug targets, phenotypic screeningand knowledge of transporters: Where drugdiscovery went wrong and how to fix it. The FEBS Journal. 2013;**280**(23):5957-5980

[6] Talevi A. Multi-target pharmacology: Possibilities and limitations of the 'skeleton key approach' from a medicinal chemist perspective. Frontiers in Pharmacology. 22 Sep 2015;**6**:205. DOI: 10.3389/fphar.2015.00205. PMID: 26441661; PMCID: PMC4585027

[7] Zimmermann GR, Lehár J, Keith CT. Multi-target therapeutics: When the whole is greater than the sum of the parts. Drug Discovery Today. 2007;**12**(1-2):34-42. DOI: 10.1016/j.drudis.2006.11.008

[8] Ashburn TT, Thor KB. Drug repositioning: Identifying new uses for existing drugs. Nature Reviews. Drug Discovery. 2004;**3**(8):673-683

[9] Novac N. Challenges and opportunities of drug repositioning. Trends in Pharmacological Sciences. 2013;**34**(5):267-272

[10] Mori A. Guanidines: Historical, Biological, Biochemical, and Clinical Aspects of the Naturally Occurring Guanidine Compounds. London: Springer; 1985

[11] Mondal S, Samajdar RN, Mukherjee S, Bhattacharyya AJ, Bagchi B. Unique features of metformin: A combined experimental, theoretical, and simulation study of its structure, dynamics, and interaction energetics with DNA grooves. The Journal of Physical Chemistry. B. 2018;**122**(8):2227-2242. DOI: 10.1021/acs.jpcb.7b11928

[12] Sterne J. Pharmacology and mode of action of the hypoglycemic guanidine derivatives. In: Campbell GD, editor. Oral Hypoglycemic Agents. New York, USA: Academic Press; 1969. pp. 193-245

[13] Bailey CJ, Turner RC. Metformin. The New England Journal of Medicine. 1996;**334**(9):574-579. DOI: 10.1056/NEJM199602293340906

[14] Muller HRH. Pharmacology of galegin. Archiv fur Experimentelle Pathologie und Pharmakologie. 1927;**125**:212-228

[15] Lamoia TE, Shulman GI. Cellular and molecular mechanisms of metformin action. Endocrine Reviews. 2021;**42**:77-96

[16] Lv Z, Guo Y. Metformin and its benefits for various diseases. Frontiers in Endocrinology. 2020;**11**:191

[17] Gurung M et al. Role of gut microbiota in type 2 diabetes pathophysiology. eBioMedicine. 2020;**51**:102590. DOI: 10.1016/j.ebiom.2019.11.051

[18] Hsu S-K et al. New insight into the effects of metformin on diabetic retinopathy, aging and cancer: Nonapoptotic cell death, immunosuppression, and effects beyond the AMPK pathway. International Journal of Molecular Sciences. 2021;**22**(17):9453. DOI: 10.3390/ijms22179453

[19] Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HAW. 10-year follow-up of intensive glucose control in type 2 diabetes. The New England Journal of Medicine. 2008;**359**(15):1577-1589. DOI: 10.1056/NEJMoa0806470

[20] Aroda VR et al. Metformin for diabetes prevention: Insights gained from the Diabetes Prevention Program/Diabetes Prevention Program Outcomes Study. Diabetologia. 2017;**60**(9):1601-1611. DOI: 10.1007/s00125-017-4361-9

[21] de Jager J et al. Long-term effects of metformin on endothelial function in type 2 diabetes: A randomized controlled trial. Journal of Internal Medicine. 2014;**275**(1):59-70. DOI: 10.1111/joim.12128

[22] Triggle CR, Ding H. Metformin is not just an antihyperglycaemic drug but also has protective effects on the vascular endothelium. Acta Physiologica. 2017;**219**:138-151

[23] Marfella R et al. Metformin improves hemodynamic and rheological responses to L-arginine in NIDDM patients. Diabetes Care. 1996;**19**(9):934-939. DOI: 10.2337/diacare.19.9.934

[24] Grant PJ. Beneficial effects of metformin on haemostasis and vascular function in man. Diabetes & Metabolism. 2003;**29**:6-44

[25] Dalan R. Metformin, neutrophils and COVID-19 infection. Diabetes Research and Clinical Practice. 2020;**164**:108230

[26] Sharma S, Ray A, Sadasivam B. Metformin in COVID-19: A possible role beyond diabetes. Diabetes Research and Clinical Practice. 2020;**164**:108183. DOI: 10.1016/j.diabres.2020.108183

[27] Bramante CT et al. Metformin and risk of mortality in patients hospitalised with COVID-19: A retrospective cohort analysis. Lancet Healthy Longevity. 2021;**2**(1):e34-e41. DOI: 10.1016/S2666-7568(20)30033-7

[28] Poly TN, Islam MM, Li Y-C(J), Lin M-C, Hsu M-H, Wang Y-C. Metformin use is associated with decreased mortality in COVID-19 patients with diabetes: Evidence from retrospective studies and biological mechanism. Journal of Clinical Medicine. 2021;**10**(16):3507. DOI: 10.3390/jcm10163507

[29] Chen Y et al. Clinical characteristics and outcomes of patients with diabetes and COVID-19 in association with glucose-lowering medication. Diabetes Care. 2020;**43**(7):1399-1407. DOI: 10.2337/dc20-0660

[30] Cheng X et al. Metformin is associated with higher incidence of acidosis, but not mortality, in individuals with COVID-19 and pre-existing type 2 diabetes. Cell Metabolism. 2020;**32**(4):537-547.e3. DOI: 10.1016/j.cmet.2020.08.013

[31] de Jager CPC et al. The neutrophil-lymphocyte count ratio in patients with community-acquired pneumonia. PLoS One. 2012;**7**(10):e46561. DOI: 10.1371/journal.pone.0046561

[32] Cameron AR et al. Anti-inflammatory effects of metformin irrespective of diabetes status. Circulation Research. 2016;**119**(5):652-665. DOI: 10.1161/CIRCRESAHA.116.308445

[33] Mor A, Petersen I, Sørensen HT, Thomsen RW. Metformin and other glucose-lowering drug initiation and rates of community-based antibiotic use and hospital-treated infections in patients with type 2 diabetes: A Danish nationwide population-based cohort study. BMJ Open. 2016;**6**(8):e011523. DOI: 10.1136/bmjopen-2016-011523

[34] Pernicova I et al. Metformin to reduce metabolic complications and inflammation in patients on systemic glucocorticoid therapy: A randomised, double-blind, placebo-controlled, proof-of-concept, phase 2 trial. The Lancet Diabetes and Endocrinology. 2020;**8**(4):278-291. DOI: 10.1016/S2213-8587(20)30021-8

[35] Chen X et al. Metformin attenuates hyperoxia-induced lung injury in neonatal rats by reducing the inflammatory response. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2015;**309**(3):L262-L270. DOI: 10.1152/ajplung.00389.2014

[36] Menegazzo L et al. The antidiabetic drug metformin blunts NETosis in vitro and reduces circulating NETosis biomarkers in vivo. Acta Diabetologica. 2018;**55**(6):593-601. DOI: 10.1007/s00592-018-1129-8

[37] Singhal A et al. Metformin as adjunct antituberculosis therapy. Science Translational Medicine. 2014;**6**(263, 263):159. DOI: 10.1126/scitranslmed.3009885

[38] Yang X, Lee HM, Chan JCN. Drug–subphenotype interactions for cancer in type 2 diabetes mellitus. Nature Reviews. Endocrinology. 2015;**11**(6):372-379. DOI: 10.1038/nrendo.2015.37

[39] Mao D et al. Risk associations of long-term HbA1c variability and obesity on cancer events and cancer-specific death in 15,286 patients with diabetes - A prospective cohort study. Lancet Regional Health – Western Pacific. 2022;**18**:100315. DOI: 10.1016/j.lanwpc.2021.100315

[40] Vancura A, Bu P, Bhagwat M, Zeng J, Vancurova I. Metformin as an anticancer agent. Trends in Pharmacological Sciences. 2018;**39**(10):867-878. DOI: 10.1016/j.tips.2018.07.006

[41] Heckman-Stoddard BM, DeCensi A, Sahasrabuddhe VV, Ford LG. Repurposing metformin for the prevention of cancer and cancer recurrence. Diabetologia. 2017;**60**(9):1639-1647. DOI: 10.1007/s00125-017-4372-6

[42] Scharping NE, Menk AV, Whetstone RD, Zeng X, Delgoffe GM. Efficacy of PD-1 blockade is potentiated by metformin-induced reduction of tumor hypoxia. Cancer Immunology Research. 2017;**5**(1):9-16. DOI: 10.1158/2326-6066.CIR-16-0103

[43] Bai M et al. Metformin sensitizes endometrial cancer cells to chemotherapy through IDH1-induced Nrf2 expression via an epigenetic mechanism. Oncogene. 2018;**37**(42):5666-5681. DOI: 10.1038/s41388-018-0360-7

[44] Mallik R, Chowdhury TA. Metformin in cancer. Diabetes Research and Clinical Practice. 2018;**143**:409-419

[45] Bodmer M, Meier C, Krähenbühl S, Jick SS, Meier CR. Long-term metformin use is associated with decreased risk of breast cancer. Diabetes Care.

2010;**33**(6):1304-1308. DOI: 10.2337/dc09-1791

[46] Wu N, Gu C, Gu H, Hu H, Han Y, Li Q. "Metformin induces apoptosis of lung cancer cells through activating JNK/p38 MAPK pathway and GADD153." Neoplasma. 2011;58(6):482-490. DOI: 10.4149/neo_2011_06_482

[47] Tseng S-C et al. Metformin-mediated downregulation of p38 mitogen-activated protein kinase-dependent excision repair cross-complementing 1 decreases DNA repair capacity and sensitizes human lung cancer cells to paclitaxel. Biochemical Pharmacology. 2013;**85**(4):583-594. DOI: 10.1016/j.bcp.2012.12.001

[48] Vazquez-Martin A, Oliveras-Ferraros C, Menendez JA. The antidiabetic drug metformin suppresses HER2 (erbB-2) oncoprotein overexpression via inhibition of the mTOR effector p70S6K1 in human breast carcinoma cells. Cell Cycle. 2009;**8**(1):88-96. DOI: 10.4161/cc.8.1.7499

[49] Vazquez-Martin A, Oliveras-Ferraros C, Del Barco S, Martin-Castillo B, Menendez JA. The anti-diabetic drug metformin suppresses self-renewal and proliferation of trastuzumab-resistant tumor-initiating breast cancer stem cells. Breast Cancer Research and Treatment. 2011;**126**(2):355-364. DOI: 10.1007/s10549-010-0924-x

[50] Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature. 2001;**414**(6859):105-111. DOI: 10.1038/35102167

[51] Dalerba P, Cho RW, Clarke MF. Cancer stem cells: Models and concepts. Annual Review of Medicine. 2007;**58**(1):267-284. DOI: 10.1146/annurev.med.58.062105.204854

[52] Ma S, Lee TK, Zheng B-J, Chan KW, Guan X-Y. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene. 2008;**27**(12):1749-1758. DOI: 10.1038/sj.onc.1210811

[53] Bao S et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006;**444**(7120):756-760. DOI: 10.1038/nature05236

[54] Phillips TM, WH MB, Pajonk F. The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. Journal of the National Cancer Institute. 2006;**98**:1777-1785

[55] Ou Z et al. Metformin treatment prevents amyloid plaque deposition and memory impairment in APP/PS1 mice. Brain, Behavior, and Immunity. 2018;**69**:351-363. DOI: 10.1016/j.bbi.2017.12.009

[56] Hardie DG. AMP-activated protein kinase: Maintaining energy homeostasis at the cellular and whole-body levels. Annual Review of Nutrition. 2014;**34**:31-55

[57] Jin Q et al. Improvement of functional recovery by chronic metformin treatment is associated with enhanced alternative activation of microglia/macrophages and increased angiogenesis and neurogenesis following experimental stroke. Brain, Behavior, and Immunity. 2014;**40**:131-142. DOI: 10.1016/j.bbi.2014.03.003

[58] Mostafa DK, Ismail CA, Ghareeb DA. Differential metformin dose-dependent effects on cognition in rats: Role of Akt. Psychopharmacologia. 2016;**233**:2513-2524

[59] Ghadernezhad N, Khalaj L, Pazoki-Toroudi H, Mirmasoumi M,

Ashabi G. Metformin pretreatment enhanced learning and memory in cerebral forebrain ischaemia: The role of the AMPK/BDNF/P70SK signalling pathway. Pharmaceutical Biology. 2016;**54**(10):2211-2219. DOI: 10.3109/13880209.2016.1150306

[60] Moore E, Mander A, Ames D, Carne R, Sanders K, Watters D. Cognitive impairment and vitamin B12: A review. International Psychogeriatrics. 2012;**24**(4):541-556. DOI: 10.1017/S1041610211002511

[61] Moore EM et al. Increased risk of cognitive impairment in patients with diabetes is associated with metformin. Diabetes Care. 2013;**36**(10):2981-2987. DOI: 10.2337/dc13-0229

[62] Koenig AM et al. Effects of the insulin sensitizer metformin in alzheimer disease. Alzheimer Disease and Associated Disorders. 2017;**31**(2):107-113. DOI: 10.1097/WAD.0000000000000202

[63] Atici Y, Baskol G, Bayram F. A new approach for the pleiotropic effect of metformin use in type 2 diabetes mellitus. Turkish Journal of Biochemistry. 2 May 2022:1-6. DOI: 10.1515/tjb-2022-0013

[64] Wu H et al. Metformin: A promising drug for human cancers (Review). Oncology Letters. 2022;**24**(1):204. DOI: 10.3892/ol.2022.13325

[65] Abdool Karim SS, Devnarain N. Time to stop using ineffective COVID-19 drugs. The New England Journal of Medicine. 2022;**387**(7): 654-655. DOI: 10.1056/NEJMe2209017