Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2019; 11(3);230-234
doi: 10.25258/phyto.11.3.19
ISSN: 0975-4873

Research Article

Antimicrobial Potential of Metformin

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Received: 25th Feb, 18; Revised 10th Jan, 19; Accepted 10th May, 19; Available Online:25th Jun, 19

ABSTRACT
Diabetes mellitus is a worldwide fast growing non infectious, metabolic disorder. Hyperglycemia of this disease favors various infections. The choice of non antibiotic, antidiabetic drug metformin to treat diabetes mellitus which has antimicrobial activity was found to decrease the incidence and the severity of infections resulting in to improved outcome. It was found that metformin has antimicrobial activity against many Gram positive and Gram negative bacteria, parasites, fungi and viruses. The most promising antimicrobial activity was found against mycobacterium tuberculosis. The possible mechanisms for this anti tubercular activity of metformin are activation of AMPK and mitochondrial ROS production, acceleration of phagosome-lysosome fusion, improved immune response, increased CD 4 and CD 8 cells, rise in mycobacteria specific interferon secretion by CD 8 cells, reduced expression of inflammatory genes. Patients of diabetes mellitus with tuberculosis who received anti tubercular treatment along with metformin as an antidiabetic drug had better prognostic outcome than the similar group of patients who did not receive metformin. Metformin was also observed to increase survival in mice having endotoxemia as a result of the inhibition of mediators of the inflammation. Thus metformin was found to have promising antimicrobial activity which needs to be confirmed by meticulously planned human studies.

Keywords: Antimicrobial, Diabetes mellitus, Metformin.

INTRODUCTION

Antimicrobial resistance is a fast growing global health issue which needs to be addressed adequately and urgently so that human population need not suffer from treatable infectious diseases[1]. To overcome this, available options are to develop newer antibiotics, use antibiotic combinations or to explore the possibility of using non antibiotic drugs for antimicrobial purpose. The existing evidence suggests that the use of non antibiotic drugs with conventional antibiotics for specific infections can prevent, delay or reverse antibiotic resistance[2].

The drugs which are not conventional antibiotics pharmacologically, do possess antimicrobial activity which are labeled as non antibiotics[3,4] Examples of these non antibiotic drugs are 1] antihistaminics like diphenhydramine[5] promethazine[6] 2] psychotropics-chlorpromazine[7] 3] antihypertensives-alpha methyl dopa, calcium channel blockers like verapamil, nifedipine, amlodipine, lacidipine and beta blockers like propranolol[8,9] 4] local anaesthetic-procaine[10] 5] proton pump inhibitors[11] and 6] antidiabetic drug-metformin[12].

Metformin [dimethylbiguanide] is a first line oral antidiabetic drug preferred in the obese diabetics. History of metformin relates to the herb Galega officinalis, also known as Goat's rue which was used in the European countries to treat hyperglycemia. From this herbal medicine, Guanidine was identified and synthesized between the year 1844-1861 which was found to lower blood glucose. Dimethylbiguanidine was synthesized in the year 1922 and was observed to lower blood glucose in animal studies. In Philippines dimethyl bigauanidine labeled as flumamine was used to treat influenza and was also tested as an antimalarial agent. In 1957 Jean Sterne published the use of metformin as an antidiabetic drug. In the Europe and UK metformin was introduced to treat diabetes mellitus [DM] in 1958.Metformin was approved in the year 1994 and was introduced in USA to treat DM in 1995.In the year 2002, Metformin was proved, to reduce the progression of pre diabetics to type 2 DM . UKPDS studies done in the year 2008 confirmed the benefit of metformin in the reduction of cardiovascular risk. In the year 2011 metformin was included in the WHO's list of essential medicines[13].

Metformin was found to be a promising antimicrobial agent in various bacterial and non bacterial infections. This drug was extensively studied against mycobacterium tuberculosis infection.

Effect of metformin on mycobacterium tuberculosis [Mtb] infection-

Tuberculosis is still a worldwide menace and a leading killer disease having very high mortality rate amounting to about 1.43 million per year[14]. Although there are reasonable advances in the treatment of tuberculosis still it poses lots of limitations due to longer duration of therapy,

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emergence of multiple drug resistant [MDR] strains and various types of drug toxicities[15,16]. This demands the need for discovery of newer drugs to treat and eradicate tuberculosis. Existing therapy targets causative pathogenic organisms only which can possibly provide the chances for the development of drug resistance. The therapeutic modulation of host cell response can help to increase the eradication of pathogens and to minimize the drug resistance, which forms the new approach for drug discovery[17,18].

Evasion of host immune responses both innate and adaptive is conducive for the growth of causative organisms which results in to the prolongation of infection[19-21]. The host cell innate response against the microbia include generation of reactive oxygen species [ROS], reactive nitrogen species [RNS] and the use of phagosomal activity or autophagy pathway which help to destroy intracellular pathogens[22,23]. The mammalian target of rapamycin [mTOR] complex 1,adenosine activated protein kinase [AMPK] and serine/threonine kinase regulates this immunity[24,25]. Disturbances in the AMPK signaling and autophagy are found to be responsible for the virulence of Mtb[26,27].

When the Mtb bacilli enter in the host cells the host defence mechanism is initiated through macrophage to achieve phagocytosis of the organisms by the help of innate immune response. Even though this phagocytosis is usually very effective in internalizing and clearing most of bacillary load, Mtb adopts various survival strategies to escape this host defense. These include 1] The inhibition of phagosome-lysosome fusion.The inhibition of the growth and killing of intracellular organism depends on the fusion of mycobacteria harbouring phagosome with lysosome[28]. 2] The inhibition of acidification of phagosome which harbor mycobacteria. Mtb interferes with the host pathway which controls vacuolar acidification essential for bacillary killing. Mtb controls phagosome acidification by targeting cytokine induced SH 2 containing protein (CISH) mediated signaling[29]. 3] The recruitment and retention of tryptophan–aspartate containing coat [TACO] protein also called as coronin-1 to phagosomes harbouring mycobacteria prevents delivery of the bacteria to lysosomes. This allows the mycobacteria to escape from the bactericidal action of macrophages[30]. 4] protection from reactive oxygen and nitrogen radicals produced by macrophages which provide hostile environment to the intracellular bacilli. Virulent bacilli produce a protein that cyclopropanates mycolic acid double bonds which reduces the susceptibility for peroxide by ten-fold[31].

Metformin, an AMPK activating antidiabetic drug[32,33] was found to inhibit the growth of intracellular Mtb, attenuate the disease related immunopathology and help in enhancing the efficacy of existing anti tubercular drugs.

Study done by Amit Singhal et al found that the treatment with metformin, attenuated the growth of intracellular M.bovis bacillus Calmette-Guerin [BCG] in human monocyte cell line and also H 37Rv strain of Mtb. Metformin also restricted the replication of intracellular MDR strains of Mtb .This effect of metformin was related with AMPK activation as it was found to be abolished by AMPK inactivation due to genetic or chemical mechanism. It was observed that metformin treatment selectively induced the mitochondrial ROS [mROS] production which was attributed to the inhibition of mitochondrial complex 1 [NADH dehydrogenase] activation [34] resulting in to acceleration of phagosome lysosome fusion which promotes bacterial killing[35,36]. Production of mROS can initiate dissipation of mitochondrial membrane potential, release of cytochrome c in to the cytoplasm and triggering of intrinsic apoptotic pathway resulting in to the cell death[37]. This study also revealed that metformin could enhance the efficacy of conventional anti TB drugs which was proved by further reduction in the bacillary load in the lungs of mice treated with the combination of INH and metformin than when mice received INH alone. The dose of metformin used in this animal study was 500mg/kg which was equivalent to human dose of 2430mg/day for 60 Kg human being .This was lesser than the maximum dose of 3gms/day which can be used in the diabetic patients[35].

Metformin was found to reduce tissue pathology related to tuberculosis. It also promoted the resolution of local pathology in the lungs and spleen along with the acceleration of bacillary clearance[38]. Percentage of lung tissue involvement due to tuberculosis was reduced significantly in the metformin treated mice as compared to untreated control animal group and also when INH was combined with metformin than when INH was given alone[35].

Metformin was also found to improve immune responses. Mice infected with Mtb when treated with metformin showed increased CD 4 and CD 8 T cells in the lungs than in untreated group. It also increased mycobacteria specific interferon secreting CD 8 cells. Increase in interferon secreting CD 8 cells was also observed in uninfected mice treated with metformin. Accumulation of both CD 4 and CD 8 cells contribute to the control of Mtb infection[35].

Metformin reduced the inflammatory responses in the infected animals. It was found that 45 out of 48 pathways related to the inflammation like activation of liver X receptor, interferon regulatory factor and pathogen recognition receptors, modulated by Mtb infection were normalized after metformin treatment .Metformin treatment also reduced the expression of genes associated with the inflammation such as IL-l β, IL-6, TNFα, chemokines like CXCL-5,CXCL-10 and monocyte chemoattractant protein MCP -1 in mouse lung. It was also observed that in the dose of 500mg/kg, metformin affected the expression of 353 genes associated with the inflammation as compared to the control group[35].

Metformin reduced the severity of tubercular infection resulting in to better clinical outcome and reduced mortality in diabetic patients with tuberculosis who were receiving metformin along with anti Tb drugs than those who were not receiving metformin.[39]The incidence of latent Tb in patients of DM which was suggested by positive T-SPOT reactivity was reduced by metformin as compared to non metformin diabetic group[35].

Observational study was done by Shrujitha marupura et al on patients of DM with tuberculosis who were receiving anti Tb drugs with oral anti diabetic therapy either metformin or other anti diabetic drugs except metformin. This revealed that, metformin provided more protective and beneficial effects regarding tuberculosis than the other anti diabetic drugs[40].

These findings related to metformin as an adjunct in the treatment of tuberculosis opens the new promising avenue for the host directed therapy which can translate in to better clinical outcome.

Effect of metformin on other infections-bacterial, parasitic and viral

Arun kumar dash et al studied the antimicrobial activity of anti diabetic drugs like metformin, phenformin and rosiglitazone against the organisms like Bacillus licentiformis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Shigella flexmeri, Bacillus subtilis, Staphylococcus aureus, and Staphylococcus epidermidis. The drug concentration of metformin selected was 50,100,200,300,400 and 500 microgram/ml. This was compared with the known antibacterial drug ciprofloxacin in the concentration of 300 and 400 microgram/ml. Both the zone of inhibition and MIC were considered parameters to determine the antimicrobial activity. Observations revealed that metformin had significant antimicrobial activity. Ciprofloxacin had almost twice the antimicrobial activity to that of metformin in the concentration of 300 and 400microgram/ml. Metformin was more effective against Shigella flexmeri and pseudomonas aerugenosa and Bacillus subtilis as compared to the other strains of organisms studied. Hence it can be inferred that metformin can be used to achieve antibacterial activity in the concentration of 400-500microgram/ml[41].

Fatema Nasrin et al studied the antimicrobial activity of metformin by disc diffusion technique. Antimicrobial activity was tested against Gram positive bacteria like Staphylococcus aureus, Bacillus megaterium, Bacillus subtilis, Bacillus cereus and Sarcina lutea; Gram negative bacteria like Salmonella typhi, Escherichia coli, Vibrio parahemolyticus and Pseudomonas aeruginosa and fungi like Candida albicans, Aspergillus niger and Sacharomyces cereveceae. Concentration of metformin used was 250 microgram/ml and 500 microgram/ml. The Kanamycin in the concentration of 30 microgram/disc was used as a positive control which has a known antimicrobial activity against Gram positive and negative organisms used for this study. The antimicrobial activity of metformin was concentration dependent against the tested bacteria and was almost half in potency as compared to kanamycin. Metformin in the concentration of 500 microgram/ml exhibited the average zone of inhibition about 12-15mm as against kanamycin which was in the range of 25-30mm. Kanamycin did not exert any antifungal activity But metformin had antifungal activity in dose dependant manner against the tested fungi[12].

Glucose mediated growth of Staph.aureus in the airway epithelium of mice was observed to be reduced after receiving metformin. Similar observations were also found in human studies[42]. Metformin reduced the bacterial load of Pseudomonas aerugenosa in the airway of diabetic mice[43].

Effect of metformin on parasitic infection-After receiving metformin, mortality rate was observed to be lowered in mice infected with trypanasoma cruzi along with the reduced level of parasites in the blood[44]. The viability of the nematode trichinella spiralis was reduced in mice who were receiving metformin. It was associated with the marked reduction in inflammatory cytokines like TNF alpha, IL-6.It also decreased the expression of vascular endothelial growth factor and COX- 2 along with the reduction in oxidative stress.[45]

Hepatitis B virus induced HBsAg was decreased by the treatment with metformin in hepatitis B virus infected patients[46]. Metformin had an impact on the prognosis of female patients infected with hepatits C virus and was found to have effect on sustained viral response[47].

Effect of metformin on sepsis-Survival rate of mice having endotoxemia was increased who were receiving metformin therapy.[48] The possible mechanism postulated was the inhibition of pro inflammatory high mobility group box 1[HMGB-1] protein which is a non histone DNA binding protein secreted by macrophages, monocytes and dentritic cells. There was also decreased expression of pro inflammatory iNOS and COX2 and their mediators like NO and PGE2[48]. It also reduced the release of TNF α and IL-6[49]. Post hepatectomy sepsis in mice was found to be reduced along with decrease in the liver damage , inflammatory mediators and the inhibition of thrombus formation when they were treated with metformin[50]. Immunomodulatory effect of metformin appears to have a potential role as antisepsis therapy which needs to be confirmed by further human studies.

Thus antimicrobial effect of metformin against various bacterial and nonbacterial infections provides a strong hope that this non antibiotic drug can be used as antimicrobial agent to treat various infections. It's role in tuberculosis appears to be promising. To substantiate this, further human studies are required to establish this drug as an adjunct along with other antibiotics. Metformin should be the preferred in all diabetics as these class of patients are more prone for infections due to underlying hyperglycemia. Metformin helps to control both, the hyperglycemia and infections.

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