The journey of metformin from glycaemic control to mTOR inhibition and the suppression of tumor growth.

Abstract

Our knowledge of the effect of metformin on human health is increasing. In addition to its ability to improve the control of hyperglycaemia, metformin has been shown to reduce the burden of ageing via effects on damaged DNA and the process of apoptosis. Studies have shown that metformin may reduce the risk of cardiovascular disease through influences on body weight, blood pressure, cholesterol levels and the progression of atherosclerosis. Studies also suggest that metformin may be beneficial for neuro-psychiatric disorders, cognitive impairment and in reducing the risk of dementia, erectile dysfunction and Duchenne Muscular Dystrophy.

In vivo and *vitro* studies have shown that metformin has anti-cancer properties, and population studies have suggested that metformin may reduce the risk of cancer or improve cancer prognosis. It is thought that it exerts its anti-cancer effect through the inhibition of the mammalian target of rapamycin (mTOR) signaling pathway.

Because of its effect on the mTOR pathway, there may be a role for metformin in slowing or reversing growth of life-threatening hamartomas in Tuberous Sclerosis Complex.

Introduction

Metformin was originally discovered from the plant *Galega officinalis*. It was used in patients with influenza and malaria. It was believed to have effects that today we would describe as bacteriostatic, antiviral, antimalarial, antipyretic and analgesic. It was found that patients with diabetes who were taking this plant had lower blood glucose. This led scientists to investigate the plant and find the glucose lowering agent. Metformin was then used to lower blood sugar. However, due to its perceived side effects it did not stay on the market continuously. In some developed countries, sanction on its use was only removed two decades ago. Currently, metformin is the most widely prescribed anti-diabetic drug in the world, and it is classed as an essential drug on the World Health Organization (WHO) list.

Our knowledge of the effect of metformin on human health is increasing, with new hypotheses and knowledge in the areas of ageing, weight control, cardiovascular disease, neuropsychiatric disorders, and others.

In addition to neuropsychiatric disorders and ageing, metformin has been shown to reduce disease progression in Duchenne Muscular Dystrophy, which is a progressive neuromuscular disorder. Most individuals with Duchenne Muscular Dystrophy become wheelchair bound in their teens. There is no effective curative treatment currently. Steroids are commonly used to minimise disease progression. However, the side effects of long term steroid use are not insignificant.

Studies have also shown that metformin has anti-cancer properties. Population studies have shown that metformin reduces the risk of cancer in individuals without cancer, and also improves prognosis in individuals suffering from cancer.

Tuberous Sclerosis Complex (TSC) is a genetic condition caused by mutations in the *TSC1* and *TSC2* genes. *TSC1* and *TSC2* are tumour suppressor genes which have an inhibitory effect on mTOR. A mutation in *TSC1* or *TSC2* leads to over-activation of the mTOR pathway, which is likely to lead to less tightly controlled cell growth. This, in turn, is associated with the growth of hamartomas in multiple organs, such as cerebral tubers,

subependymal giant cell astrocytoma (SEGA), kidney angiomyolipomas (AML) and facial angiofibromas. Because of its effect on the mTOR pathway, we investigated metformin in TSC in a randomised double blind placebo controlled trial. We demonstrated that metformin is safe and well tolerated in children and adults with TSC. Patients on metformin had a significant reduction in SEGA volume compared with placebo. Metformin did not reduce AML size but growth appeared slower than in the placebo group, although this difference was not statistically significant. There may be a role for metformin in slowing or reversing the growth of life-threatening hamartomas in TSC.

In this review we demonstrate that metformin, which was discovered more than a century ago for lowering blood sugar, has significant potential in non-curable and progressive conditions. More than 90% of the world's population live in poverty, where patients do not have access to high cost therapies. Metformin is cheap and safe, making it a more viable option than many other therapies.

Metformin beyond control of blood sugar

Metformin and ageing

It has been postulated that metformin reduces the burden of ageing. Two large clinical trials the Investigation of Metformin in Pre-Diabetes on Atherosclerotic Cardiovascular OuTcomes (VA-IMPACT) and the Targeting Aging with Metformin (TAME) are aiming to assess the effects of metformin in non-diabetic patients, and particularly its effects on the ageing process.

The process of ageing is complex but a crucial element is DNA damage and cell death, processes which are initiated via mechanisms that include Inflammatory markers including interleukins.

Metformin inhibits the translocation of the transcription factor, NF-kB to the nucleus and prevents the phosphorylation of IκB and IKKα/β, which are required for activation of NF-κB pathway.(1) Activation of NF-κB pathway leads to production of inflammatory cytokines. It has been suggested that chronic inflammation leads to aging and this phenomenon is known as inflammaging. Metformin exhibits its antiaging effect via the inhibition of the NF-κB pathway. (1)

Metformin can also minimise the production of reactive oxygen species (ROS) in respiratory chain complex 1 which prevents oxidative stress related cell death. ROS are an endogenous source for DNA damage. (2)

Ceramides are found within cell membranes and can play a role in cellular signaling, including cell differentiation, proliferation, and programmed cell death. Ceramides are believed to have a role in the ageing process through the cell death programme. They inhibit myoblast proliferation and cell cycle regulation in skeletal muscles. Metformin has been shown to minimise the harmful effect of ceramides. (3)

Metformin may also have a role in cardio-protection via its effect on the endothelial nitric oxide synthase dependent pathway which can stimulate ischemia induced revascularization. It can also be neuroprotective, reducing neuronal damage by preventing etoposide-induced apoptosis in neurons (4, 5)

Metformin and cardiovascular disease

Human studies have shown that metformin reduces the risk of cardiovascular disease in diabetic patients. The cardiometabolic effect has also been evaluated in diabetic patients. Studies have shown that those individuals who were taking metformin had a reduction in body weight, LDL cholesterol, and atherosclerosis, based on carotid artery intima-media thickness analysis.(6, 7)

Metformin and weight loss

Studies have shown that individuals with diabetes, without diabetes, and those at risk of diabetes, are likely to lose a small but beneficial amount of weight when taking metformin. Metformin has not been approved for weight loss but some clinicians use it in over-weight patients who are at risk of diabetes.(8-10)

Metformin and neuropsychiatric disorders

Depression

A positive effect of metformin in depression has been reported. Guo et al reported that patients with type 2 diabetes who were on metformin showed improvement in depression. This is may be because these patients achieved better glycemic control.(11)

Cognitive abilities

Studies have shown that individuals who take metformin are likely to show a reduction in mild cognitive impairment compared with those patients who are not taking metformin, or those taking other hypoglycemic agents. The risk of dementia has also been reported to be lower in those who take metformin. (12, 13)

One could argue that there are a lot of cofounding factors with these patients such as cardiovascular disease, age, diabetes, and other comorbidities. However, this study by Luchsinger et al has suggested that metformin also improves cognition in individuals without diabetes.(14)

Metformin and erectile dysfunction

There are three possible mechanisms involved in the pathogenesis of erectile dysfunction such as endothelium-dependent vasodilatory impairment, sympathetic nerve activity elevation and atherosclerotic luminal narrowing. Metformin exerts its effect via these pathways. Animal studies have shown that treatment with metformin in rats and rabbits restores the transcription of endothelial nitric oxide production in the penile tissue, thus improving the endothelium dependent vasodilatory impairment. (15)
Metformin has also been shown to improve the endothelia dependant vasodilatation in diabetic and non diabetic patients. (16) Sympathetic over-activity is another possible mechanism for erectile dysfunction. Metformin has been shown to reduce the level of norepinephrine which is a marker of sympathetic activity. (17)

Atherosclerotic luminal narrowing is another possible mechanism for erectile dysfunction which is linked to high blood pressure. Metformin has been shown to attenuate hypertension, thus improving or reducing the occurrence of atherosclerotic luminal narrowing. (18)

Metformin and Duchenne Muscular Dystrophy (DMD)

DMD is an X-linked recessive neuromuscular disorder that affects 1 in 3,500–6,000 male births. Generally, children with DMD present in early childhood with proximal muscle weakness and become wheelchair-dependent by adolescence. This is a progressive muscle degeneration disorder which is caused by a mutation in the *DMD* gene. Mutation in this gene leads to an absence of the protein dystrophin. (33, 34) Dystrophin is located primarily in skeletal and heart muscle, where it helps stabilize and protect muscle fibers. Loss of dystrophin leads to loss of cytoskeletal integrity.(35) This in turn leads to dysregulation of calcium homeostasis and increased production of reactive oxygen species (ROS), which results in protein and membrane damage. Mitochondria are the main source for cellular ROS. High production of cellular ROS implies altered mitochondrial function in DMD. (36) Biopsy samples of DMD patients have shown reduced rates of cellular respiration and lower activities of enzymes of the mitochondrial respiratory chain. (37) These findings have also been seen in DMD mouse models. (38)

Loss of dystrophin in DMD is associated with a significant reduction in neuronal nitric oxide (NO) activity. (39) NO activation is essential for mitochondrial function in order to minimise oxidative stress and to improve fat usage for energy production. (40) NO plays a role in regulating muscular energy. Activation of AMP-activated protein kinase (AMPK) stimulates NO synthesis. (41) Animal studies have shown that in DMD animal models, activation of AMPK reduces muscle fatigability and improves muscle functions. (42)

Hafner et al used metformin 250mg twice a day with L-arginine for 16 weeks in five ambulatory children with DMD. The authors reported no serious side effects and none of the patients dropped out of the study. It was noted in the muscle biopsy samples before treatment that there was a significant reduction in the mitochondrial protein expression and an increase in oxidative stress. They reported that there was a significant increase in the mitochondrial electron transport chain and a reduction in oxidative stress after treatment. They also reported a reduction in resting energy expenditure rates, and energy substrate use shifted from carbohydrates to fatty acids. It was concluded that pharmacological stimulation of the nitric oxide pathway with metformin leads to an improvement in mitochondrial function and a slowing of disease progression. (43)

Metformin and Cancer

Population studies have suggested that metformin may reduce the risk of cancer or improve cancer prognosis. This led scientists to further investigate metformin. (19, 20)

Vivo and in vitro studies have shown that metformin has anti-cancer properties. The mechanism by which metformin exhibits its anti neoplastic effect is through the inhibition of the mammalian target of rapamycin (mTOR) signaling pathway, by activating the AMPK regulator (adenosine monophosphate-activated protein kinase) and p53.

In vitro experiments have been carried out to investigate the effect of metformin on epithelial cells in breast cancer cells. The aim was to ensure that this effect is a direct effect of metformin rather than through insulin levels. It has been noted that metformin acts as a growth inhibitor for MCF-7 human breast cancer cells. MCF-7 is the acronym of Michigan Cancer Foundation-7. These cell lines were discovered in 1970s. These cells are known to be responsive to insulin. The effect of metformin has been through the suppression of phosphorylation of p70S6K at Thr389 rather than through insulin or insulin growth factors (IGF). Activation of the AMPK pathway by metformin is observed in epithelial breast cancer cells. Activation of AMPK leads to inhibition of the mTOR pathway, reduction in proliferation, S6 kinase inactivation, reduction in mRNA translation and protein synthesis in these cancer cells. (21)

Other studies have investigated the effect of metformin on other cancer cells such as colon cancers. A group studied the effect of metformin on human colonic carcinoma cell lines. They used paired isogenic human colonic carcinoma cell lines, HCT116 p53+/+ and p53−/− in nude mice. The right flank of the mice was injected with p53−/− cells and the left flank with HCT116 p53+/+. The animals were given intraperitoneal metformin or saline solution, four days post cell injection. It is known that loss of wild-type p53−/− accelerates tumor formation in untreated animals. The group reported a significant tumor volume reduction in the metformin group in the HCT116 p53−/− cells compared with the growth of the tumors from p53+/+ cells in the opposite flank. The tumor volume after one month for the p53−/− xenografts was >50% smaller than p53−/− xenografts from the animals treated with saline solution. (22)

Another group evaluated the effects of metformin on renal cell carcinoma and its main mechanisms. They reported that metformin was able to cycle arrest and inhibit renal cell carcinoma growth in vitro and vivo via the activation of AMPK, and inhibition of mTOR signalling in renal cell carcinoma. They investigated the effect of metformin on cancer cell proliferation by treating 786-O and OS-RC-2 renal cell carcinoma cells with different metformin concentration. It was noted that metformin was able to significantly inhibit the proliferation of renal cell carcinoma. They also observed that metformin is able to prevent cell colony formation of the renal cell carcinoma cells. (23)

Metformin has also been shown to inhibit cell proliferation and migration in the oral squamous cell carcinoma cell line model. It has been reported that metformin reduces HIF-1a mRNA and protein levels. It has also been observed that metformin increases the level of PDH (Pyruvate Dehydrogenase) in hypoxic conditions. It is believed that metformin has an anti-proliferative effect, and can inhibit migration in squamous cell carcinoma. In addition, it increases the number of apoptotic cells and the transcription of caspase 3. (24)

Scientists have attempted to use nano particles to deliver metformin to cancer cells. It is an attractive idea as these particles can potentially deliver drugs directly to the affected tissue, in high doses, without systemic adverse effects. (25) Snima et al developed nano particles which contained metformin, and assessed the effect of metformin on both a pancreatic cancer cell line and a normal cell line. The nano particles were measuring 240 SD 50 nm and were made through the ionic-gelation method. This consisted of a coat of O-carboxymethyl chitosan which easily incorporated metformin molecules because of the electrostatic attraction between the carboxymethyl negative charges of the chitosan derivative and the NH4 positive charges of metformin molecules. The degree of release of metformin from the nano particles was pH dependant. An acidic environment caused faster release of the drug than an alkaline environment. As tumour environment is more acidic, it may retain and attract more metformin.(26) The long term safety of these particles are unknown. The particles may change the permeability of red cell membranes, by forming conduction pores or by modifying the activity of sodium/potassium or calcium/magnesium pumps, and therefore their safety may be questionable. (27) Metformin containing nano particles were shown to be

haemocompatible in Wistar rats and the particles had a haemolytic ratio of less than 5 %, thus confirming their safety in the case of oral administration (27). In addition, the kidney and liver function of the rats remained unchanged in spite of the presence of the particles in the kidney and liver tissue.(28)

Metformin and cancer in human studies

Human studies have shown that metformin may aid cancer prevention and possibly avoid tumour recurrence. A large study tested the hypothesis that metformin reduces the risk of cancer in people with type 2 diabetes. This was an observation cohort study in Scotland. Patients with type 2 diabetes who were taking metformin from 1994-2000 were identified. These patients were compared with another group of diabetic patients who were not taking metformin. The groups were matched by year of diabetes diagnosis. They investigated the ratio of cancer diagnosis in the two groups. It was noted that cancer was seen in 7.3% of 4,085 metformin users compared with 11.6% of 4,085 diabetic patients who were not receiving metformin. They reported an unadjusted hazard ratio (95% CI) for cancer as 0.46 (0.40-0.53). The ratio was adjusted for several cofounding factors such as sex, age, BMI, Hb A1C, deprivation, smoking, and other drug use, and reported it to be 0.63 (0.53-0.75). The authors concluded that metformin may have a role in reducing the risk of cancer. (29)

Another study investigated the link between tumour complete response rate and metformin in diabetic patients with breast cancer who were receiving neoadjuvant chemotherapy. The Authors identified 2,529 patients who were given neoadjuvant chemotherapy for breast cancer from 1990 and 2007. The group consisted of 68 diabetic patients who were receiving metformin, 87 diabetic patients were not receiving metformin and 2,374 nondiabetic patients. The complete response rate was noted to be 24% in the metformin group, 8.0% in the group who were not receiving metformin, and 16% in the nondiabetic group (p= .02). This study concluded that diabetic patients who had breast cancer and were receiving metformin had a higher complete tumour response rate than the other group. (30)

Another study investigated the effect of metformin in diabetic patients who had colorectal cancer. In this study, 86 patients with colorectal cancer had diabetes. This group was divided into two groups, metformin and non metformin. It was noted that metformin enhanced the anti-proliferative effects of 5-fluorouracil on CD133+ in cancer stem cells. It was reported that the distant metastasis rate in the patients receiving metformin was significantly lower than in the non-metformin group (5.60% vs 21.6%, p=0.035). In addition, less patients in the metformin group had differentiated adenocarcinoma than in the other group (2.78% vs 16.0%, p=0.048). These results supported the results of the previous studies. Better outcomes of patients with colorectal cancer on metformin was contributed to the inhibitory effect of metformin on CD133+ colonic cancer stem cells.(31)

Figure 1: Metformin and the mTOR pathway –This is a reproduced diagram. The original diagram was created by Pernicova et al. Written permission has been obtained from the senior author, Dr Korbonits. (32)

Metformin and mTOR pathway

The exact mechanism of action of metformin is not well understood. As illustrated in figure 1, metformin can exert its inhibitory effect on the mTOR pathway. (44)

Both complexes mTORC1 and mTORC2 contain a catalytic subunit mTOR. These two complexes are crucial for cellular growth and receive stimuli from various energy and hormonal signaling.

mTORC1 receives signals from different pathways such as insulin, IGF1 (Insulin like Growth Factor 1), IGF2 and via AMPK (AMP-activated protein kinase) as shown in figure 1. (45)

Inhibition of mTORC1 by metformin via AMPK pathway is through the activation of tumour suppressor genes *TSC1* and *TSC2* genes which code for tuberin and hamartin protein respectively. In addition, metformin can inhibit mTORC1 directly via AMPK and this is achieved by AMPK inhibiting RAPTOR (regulatory-associated protein of mTOR). AMPK phosphorylates RAPTOR and this phosphorylation is required for the inhibition of mTORC1 complex. RAPTOR is an adaptor protein and positive regulator within the mTORC1 complex. (46)

Metformin can also inhibit mTORC1 via IGF-1 and insulin signaling pathway. In order to promote cellular growth, both insulin and IGF 1 block the TSC1 and TSC2 which lead to activation of mTORC1. Blocking IGF and insulin by metformin, means that TSC1 and TSC2 can exert their inhibitory effect on mTORC1. (47)

Metformin can also induce p53. p53 is a tumour suppressor protein that can inhibit mTORC1. p53 can sense genotoxic stresses such as DNA damage which could change the genetic material of cells which could then stimulate p53 in order to stop cellular

growth and proliferation. p53 activates AMPK, and subsequently TSC1 and TSC2, which then inhibit mTORC1. The activation of AMPK by p53 is by formation of a complex, LKB-1-p53. This complex has been shown to regulate and activate AMPK. (48)

Metformin increases the expression of *DICER1* gene which codes for the DICER enzyme. It is an RNase III family. It cleaves double-stranded RNA and pre-microRNA into short double stranded RNA fragments which are microRNA and small interfering RNA. Mutation in *DICER1* gene leads to a complex tumour syndrome. Hence metformin has an antineoplastic effect via induction of DICER expression. (49)

Metformin has also been shown to inhibit the proto-oncogene *c-MYC*. *c-MYC* is overexpressed in many cancers. It plays a crucial role in growth control, differentiation and apoptosis. (50)

Metformin is also an inhibitor of HIF-1α (hypoxia inducible factor) via AMPK and mTORC1. HIF-1α is an oxygen sensitive transcriptional activator which mediates the tissue response to low oxygen. It facilitates adaptation and survival of cells during changes in oxygen levels. It also has a key role in metabolic transformation in cancer. (22, 24)

The drug metformin has also been shown to inhibit the expression of fatty acid synthase, which is a multi-enzyme protein that catalyzes fatty acid synthesis. Fatty acid synthase has been shown to be an oncogene and is upregulated in cancer cells. (51, 52) Growth factors and amino acids activate mTORC1 through the RAG GTPases which is the Ras-related GTPases, independently of AMPK. Activation of the RAG GTPases by the Ragulatory complex leads to the mobilization of mTORC1 to the lysosomal surface. The complex is then activated by RHEB. Metformin can also inhibit mTOR complex 1 through direct inactivation of the Ragulator complex, which will inhibit RAG GTPases, leading to dissociation of mTORC1 from its activator Rheb.(50, 53)

Metformin reduces production of ROS (reactive oxygen species), oxidative stress and DNA damage through the inhibition of mitochondrial complex I. (2, 24, 54)

It has been postulated that metformin inhibits ATM, serine-protein kinase. This is because it has been noted that variation in glycemic control in patients with type 2 diabetes has been linked to the presence of common genetic variants adjacent to the ataxia telangiectasia mutated *(ATM)* gene. Ataxia telangiectasia is a neurological condition caused by mutation in the *ATM* gene. Patients with this condition are susceptible to cancer and type 2 diabetes. The *ATM* gene encodes for a tumour suppressor protein which is important for DNA repair and cell cycle control. (55)

Metformin in animal TSC models

The efficacy of metformin has been trialed in TSC animal models. $Tsc2^{+/-}$ mice models can develop renal cystadenomas which increase in size as the animal gets older. The lesions can progress to renal cell carcinoma. These murine models of *Tsc2* were developed by using a gene targeting approach. It has been shown that *Tsc2–/–* is embryonic lethal at days 9.5–12.5 from hepatic hypoplasia. *Tsc2+/–* mice develop a wide variety of neoplastic growths.

These lesions grow slowly and are less likely to become malignant neoplasms. The group showed that a 15 month old *Tsc2+/–* has approximately 100 cystadenomas lesions in each kidney. Renal cell carcinoma was seen in 3 animals out of 150 at the age of 12 months. Only one had lung metastasis. All the other growths in the lung, liver and limbs show a slow growth rate.(54)

The authors claimed that this model may be better than the previously described TSC animal models as the gene disruption is better characterized in these models. The range of growths that have been seen in the $Tsc2^{+/–}$ mice differs from that in patients with TSC. However, the slow growth rate and the limited change to malignant neoplasm make these models suitable as TSC related lesions in patients with TSC behave similar to these.

The frequency of kidney cystadenomas in these models is not dissimilar to the Eker rat *Tsc2+/-*. However, Eker rats develop haemangiomas in their spleen and uterus, rather than liver. (55) In addition, pituitary tumors and cerebral hamartomas are seen in Eker

rats, whilst *Tsc2*$^{+/-}$ mice don't tend to develop these lesions. The rats can also have lung lesions. The scientists suggest that the differences in phenotype between the mice and rats is probably due to the difference in their genetic make up rather than due to differences in the way the genes are expressed or the function of tuberin. (56)

A group in Boston investigated the effect of metformin in *Tsc2*$^{+/-}$ A/J mice on renal cystadenomas. A/J mice are commonly used to model cancer as they have high susceptibility to carcinogen induced tumours. These animals were given one of five treatment regimens. The first group of 9 mice were given rapamycin intraperitoneally at 6mg/kg for 3 days per week. The second group of five mice were given vehicle on the same schedule. In the third group, Bortezomib was given to 8 mice at 0.8 mg/kg subcutaneously for two days per week. Bortezomib is a proteasome inhibitor which aggravates ER (endoplasmic reticulum) stress in cancer cells lacking *TSC* genes. It has been approved for multiple myeloma. It is believed that this drug kills the myeloma cells via the induction of apoptosis.(57, 58)

In the fourth group, metformin was given in 5% sucrose drinking water at 300 mg/kg per day to 10 mice. The fifth group of 8 mice were given 5% sucrose drinking water as a control group.

The first three groups were treated simultaneously, as were the last two groups. Rapamycin was only given for one month as it has been shown in a previous study to be effective in reducing tumor volume in Tsc2$^{+/2}$. (59)

Bortezomib was also given for month because of its potential significant side effects and toxicity. Metformin was given for 4 months. The dose of metformin was obtained from a study which investigated the effect of metformin on prevention of tumours in a combined LKB1-PTEN mouse model. (60)

The animals were killed at the age of 5 months. Rapamycin and Bortezomib were given to mice aged 4 months. Whilst metformin was given to mice aged 1 month.

The rapamycin group showed a significant difference in the tumor extent before and after treatment compared with the vehicle and other treatment group. This was assessed by gross observation. Based on the microscopic assessment, the rapamycin group showed a significant difference in tumour extent when compared with the vehicle group and the other treatment groups.

Whilst the tumour volume per kidney was not significantly reduced for the other two treatment groups, bortezomib or the metformin group. Both bortezomib and metformin showed pharmacodynamic effects as the authors expected. The authors concluded that neither bortezomib nor metformin have a significant benefit in the native $Tsc2^{+/-}$ mouse model. This suggested that these treatment options may have limited benefits in treating TSC related hamartomas. (61)

Shen et al investigated the effect of metformin on kidney lesions in a $Tsc1^{+/–}$ mouse model of tuberous sclerosis complex. In this study, the mice were randomly allocated to either metformin or drinking water. There were 10 mice in each group. The treatment baseline was commenced at 6 months and stopped at 15 months of age. 150mg/kg of metformin was given in the first 7 months and then increased to 600mg/kg for 2 months. The mice had MRI scans at the end of the trial. Interestingly the weight change was not significant between the groups. The mean weight gain for the treatment group and placebo were +2.26g and +2.30g respectively. MRI scans were used to assess the renal lesions. There was no significant difference between the total number of kidney lesions for each mouse, or the number of type specific renal lesions, such as cysts, papillary or solid lesions. In addition, the mean and individual volume of renal lesions were not significantly different between the groups. They also examined the activity of the mTOR pathway in the tissue samples. The level of pS6 (Ser235/236) was noted to be marginally lower in kidney tissues of those animals who received metformin. However, there was no consistent difference in pS6 (Ser235/236) level in tumour cells between the control and treatment groups. They also investigated the phosphorylation status of other metformin targets such as pAMPK (Thr172) , pACC (Ser79), pAkt (Ser473) and pRaptor(Ser792). There was no noticeable effect of metformin on the phosphorylation level of pAkt(Ser473) or pRaptor(Ser792) in Tsc1+/-mouse kidney tissues.

The authors have also studied the essential transporter for metformin uptake. SLC22A2 is a crucial organic cation transporter in the kidneys. It was noted that this transporter was significantly reduced in renal tumor cells compared with normal renal cells in both Tsc1+/ and Tsc2 +/-mice. It was concluded that metformin reduces mTORC1 signalling in Tsc1+/-normal kidney tissue but not in the tumour cells. The lack of efficacy of metformin may be due to the suppression of the expression of organic cation transporters such as SLC22A1, SLC22A2 and SLC22A3.(62)

Metformin in humans with TSC

TSC is a relatively common genetic disorder with a prevalence of 4–9 per 100,000. (63) (64) It is characterized by the development of tumours (hamartomas) throughout the body. Hamartomas can occur in almost any tissue but particularly in the kidneys, brain, skin and heart. TSC is associated with difficult epilepsy (approximately 75% of patients); learning difficulties (approximately 50%, 30% with profound learning disability IQ<21); (63) and a range of psychological and behavioral problems including autism. (65, 66)

Tumours on the skin and nails can be significantly disfiguring. Many adults with TSC are unable to live independently and require state or family care. (67) Tumours affecting the heart (68) kidneys (69) and brain can cause life- threatening complications. (70)

Kidney tumours tend to increase in number and size with increasing age, and can be associated with symptomatic bleeding, sometimes life- threatening, in 10%. Patients are likely to require lifelong health-care follow-up.

The two genes responsible for TSC (called *TSC1* and *TSC2*) were identified in the 1990s. Since then there has been considerable progress in elucidating the molecular mechanisms by which they exert their influence. [103] They play a central role in regulating an insulin driven cell signalling pathway (IRS-PI3Kinase-mTOR-S6 Kinase) that promotes cell growth. (71) Mutations in *TSC1/2* mean that their gene products do not inhibit mTOR effectively. This allows the promotion of unregulated cell growth that leads to the development of tumours. It is postulated that drugs that inhibit mTOR will inhibit or reverse tumour development in TSC. (72)

Rapamycin and everolimus are drugs that are known to inhibit mTOR and recent studies have shown that they can reduce TSC-related lesions such as kidney AMLs, brain SEGAs and facial angiofibromas (73-77). Spontaneous regression of these hamartomas is not reported. (76, 78, 79)

A recently published Phase III trial has shown that everolimus, when used as an adjunctive therapy, significantly reduced treatment-resistant focal seizures in individuals with TSC compared with placebo. 64% of patients who were on everolimus had stomatitis compared with 9% on placebo. Diarrhoea was reported in 22% vs 5% and hypercholesterolaemia in 7% vs 1%. (77)

In a phase III (EXIST 1) international, multicentre, double-blind, randomized, placebo-controlled trial, Franz et al evaluated the efficacy and safety of everolimus in patients with SEGA. Everolimus was associated with a significantly greater overall SEGA response rate (>/= 50% shrinkage), compared with placebo (35% vs. 0%). The most common adverse events were mouth ulceration (25 [32%] in the everolimus group *vs* two [5%] in the placebo group), stomatitis (24 [31%] *vs* eight [21%]), convulsion (18 [23%] *vs* ten [26%]), and pyrexia (17 [22%] *vs* six [15%]).(80)

Exist-2 was a placebo controlled phase 3 randomized controlled trial investigating the efficacy and safety of everolimus in treating renal AMLs > 3cm in adults. (81) The angiomyolipoma response rate (Defined as >/= 50% shrinkage) was 42% (33 of 79 [95% CI 31–53%]) for everolimus and 0% (0 of 39 [0–9%]) for placebo. The most common adverse events in the everolimus and placebo groups were stomatitis (48% [38 of 79], 8% [3 of 39], respectively), nasopharyngitis (24% [19 of 79] and 31% [12 of 39]), and acne-like skin lesions (22% [17 of 79] and 5% [2 of 39]). (82).

Metformin is a drug that potentially offers the benefit of mTOR inhibition without the side effect and cost profile of other mTOR inhibitors. It is used by millions of people with type 2 diabetes and has a benign side effect profile. (83)

We treated 51 TSC patients with either placebo or metformin in a multi-centre randomized, double-blind, parallel group, placebo-controlled trial of metformin. The patients were treated for 12 months. The mean kidney (angiomyolipoma) AML volume increase from baseline was 25.5% for the placebo group and 9.6% for the metformin group. Difference in response, 15.9% (95% CI -9% to 41%) p=0.221. The mean SEGA volume increased from baseline by 37% in the placebo group but reduced from baseline by 23.3% in the metformin group. Difference in response, 60.3% (95% CI -0.4% to 111, p=0.048). We reported three serious adverse events that reflected the underlying disease. We concluded that metformin is safe and well tolerated in children and adults with TSC. Patients on metformin had a significant reduction in SEGA volume compared with placebo. We also saw seizure reduction in the metformin group. The growth of AML in the treatment group was slower than in the placebo group. (84)

Conclusion

In addition to glycaemic control, metformin has multiple affects, and exerts its function via several cellular pathways. Metformin positively affects the ageing process, minimises the risk of cardiovascular disease, has an anti-inflammatory effect, improves cognition, improves neuropsychiatric disorders and has anti cancerous properties. It can cause small beneficial weight loss. Metformin may also have a role in slowing the progression of Duchenne Muscular Dystrophy. In addition, there may be a role for metformin in slowing or reversing the growth of life-threatening hamartomas in TSC. Given its long-term safety and cost profile, it may become a pharmacological intervention in many non diabetic-related conditions.

Table 1: shows a summary of the current and potential uses of metformin in clinical practice.

Current use of metformin
• Type 2 Diabetes mellitus • Poly cystic ovary disease
Potential use of metformin
• Cardiovascular disease • Weight loss • Depression • Cognitive abilities • Erectile dysfunction • Anticancer • Anti ageing • Tuberous Sclerosis Complex • Duchenne Muscular Dystrophy

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