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A marriage of two "Methusalem" drugs for the treatment of psoriasis?

Arguments for a pilot trial with metformin as add-on for methotrexate

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Abbreviations: AICA, 5-aminoimidazole-4-carboxamide; AICART, AICAR transformylase; AICAr, 5-aminoimidazole-4-carboxamide ribonucleoside; AICAR, 5-aminoimidazole-4-carboxamide ribonucleotide, synonymous with ZMP; AICART, AICAR transformylase; AMPK, AMP-activated protein kinase (Thr/Ser); DPP, Diabetes Prevention Program; IL, interleukin; LDL, low density lipoprotein; LPS, lipopolysaccaride; MATE, multidrug and toxin extrusion proteins; mTORC1, Mammalian Target of Rapamycin Complex I; MTX, methotrexate; MTXPG, methotrexate polyglutamate; OCT, organic cation transporter; PASI, Psoriasis Area and Severity index; PCOS, polycystic ovary syndrome; PTP, permeability transition pore; ROS, reactive oxygen species; TLR, toll like receptor; TNF, tumor necrosis factor; PTP, permeability transition pore

In this article we present arguments that the "antidiabetic" drug metformin could be useful as an add-on therapy to methotrexate for the treatment of psoriasis and, perhaps, for rheumatoid arthritis as well. Biochemical data suggest that both drugs may share a common cellular target, the AMP-activated protein kinase (AMPK). This enzyme is a master regulator of metabolism and controls a number of downstream targets, e.g., important for cellular growth or function in many tissues including T-lymphocytes. Clinical observations as well as experimental results argue for anti-inflammatory, antineoplastic and antiproliferative activities of metformin and a case-control study suggests that the drug reduces the risk for psoriasis.

Patients with psoriasis have higher risk of metabolic syndrome, type 2 diabetes and cardiovascular mortality. Metformin has proven efficacy in the treatment of prediabetes and leads to a pronounced and sustained weight loss in overweight individuals. We expect that addition of metformin to methotrexate can lead to positive effects with respect to the PASI score, reduction of the weekly methotrexate dose and of elevated cardiovascular risk factors in patients with metabolic syndrome and psoriasis. For reasons explained later we suggest that only male, overweight patients are to be included in a pilot trial. On the other side of the coin are concerns that the gastrointestinal side effects of metformin are intolerable for patients under low dose, intermittent methotrexate therapy.

Metformin has another side effect, namely interference with vitamin B_{12} and folate metabolism, leading to elevated homocysteine serum levels. As patients must receive folate supplementation and will be controlled with respect to their B_{12} status increased hematological toxicity is unlikely to result.

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Introduction

It is an undeniable fact that there is a paradigmatic change occurring for one of the most common "skin diseases," psoriasis. It is now more and more recognized as a systemic disorder.[1] Being asked to prepare state-of-the-art review talks (pharmacological and dermatological, respectively) for a conference held in Vienna on behalf of the 50th anniversary of methotrexate we discovered a case-control study indicating that risk for psoriasis was lowered by using either glitazones or metformin.[2] Risk reduction for metformin was only observed in males. The authors suggested that, perhaps a common mechanism, namely activation of AMP-activated protein kinase (AMPK) was a possible explanation. Although the glitazones can stimulate AMPK in cellular systems after binding to a mitochondrial receptor (mitoNEET)[3] AMPK activation was never (in contrast to metformin) demonstrated ex vivo in humans. Association studies do not prove causality but can stimulate further research. Disregarding the glitazones for reasons not detailed here, we concentrate on metformin to collect arguments for a pilot trial adding this "antidiabetic" as an "anti-inflammatory" agent to the low-dose, weekly methotrexate regimen.

Dermatologists are familiar with methotrexate but less so with metformin, therefore the focus is more on the latter.

Endocrinologists on the other hand may be less informed about the biochemical mysteries of the most favored "anti-diabetic" drug. Metformin was introduced in Europe during the same decade when Gubner et al.[4] published their results with aminopterin for the treatment of psoriasis and rheumatoid arthritis. First we discuss pharmacodynamics and molecular and cellular activities. A brief overview on the cellular and organismal distribution and disposition-kinetics of the two drugs is also given. We subsequently present biochemical data, animal experiments and clinical observations for our proposal that an "add-on" pilot trial with male psoriasis patients having metabolic syndrome is a rational approach and ethically justified. It must be emphasized that this is not a review article on anti-inflammatory activities of metformin (although we heavily rely on existing evidence) or of methotrexate. Discussions of complex intracellular pathways (including signaling of cytokines, protein kinase cascades, etc.) are beyond our scope. For readers interested in more molecular details we cite key review articles and publications. It is hoped—although both drugs are old (but by no means outdated)—that what was selected from the most recent and long forgotten literature can be fascinating. Perhaps we can stimulate interest of researchers and clinicians in dermatology for metformin and related compounds as systemic (as proposed here for psoriasis) or even as topical drugs.

Results

Methotrexate and metformin are not only very old drugs but have a remarkable history of an expanding pharmacodynamic profile. *Metformin: From an anti-diabetic to anti-inflammatory, anti-aging, rejuvenating, antidote, anti-neoplastic and "anti-androgen" drug.* The history of the biguanidines as drugs can be traced back to the Middle Ages. The herb *Galega officinalis* (French lilac, Goat's rue) was used among other for symptoms of diabetes mellitus in humans or to increase milk production in farmed animals. The active principles of the toxic (or poisonous) plant are guanidine and isoamylene guanidine (galegine) recognized as hypoglycemic principles early in the 20th century.[5] Galegine was used in doses of 150 mg/day with success in more than 3 dozen patients, lacking the abdominal discomfort of synthetic synthalin A (decamethylene-guanidine) and B (dodecamethylene diguanidine).[6] The synthalins remained in the pharmaceutical armamentarium of Germany for many years despite their known toxicity. Under the suggestive name of flumamine, injected (most likely i.m. in a dose of 32.5 mg!) metformin enjoyed a brief episode in 1949 as an extremely potent, fast-acting antipyretic and analgesic for viral influenza.[7,8] Although this miracle cure was never reproduced, the author suggested that flumamine acts on malaria parasites and promised to report on his malaria cases later. His idea that metformin (and related biguanidines) may be active on malaria parasites is supported by the structural similarity of RJF 00719 [2-(4-cyclohexylphenyl)-1-diaminomethylidene-guanidine)] and RJF 01059 (phenylbiguanide) to phenformin. Both compounds (especially RJF 00719 in the 20 micromolar range) are inhibitors of the *Plasmodium falciparum* bi-functional thymidilate synthase-dihydrofolate reductase.[9]

In any event, the publication[8] from the Philippines, mentioning glucose lowering effects of substituted biguanidines in the discussion (but not for his treated patients), stimulated a French researcher (Jean Sterne) 1957 to try "flumamine" for diabetics. Jean Sterne was (luckily) unaware of data published a quarter of a century earlier in the at that time most important pharmacological journal of the world.[10] The latter German authors, who investigated the antipyretic (sic) activity of biguanide and its chemically modified analogs, actually strongly warned against a human trial for diabetes: 1,1-dimethylbiguanide (now metformin) was extremely toxic in their animal experiments. In any event, the above mentioned "miracle" cure was the first hint that metformin and structurally related antidiabetics such as phenformin can exhibit "anti-inflammatory" activity. Indeed, in the pre-methotrexate era phenformin was tried with some success for rheumatoid arthritis because it demonstrated "fibrinolytic" activity.[11-13] After the discontinuation of phenformin and buformin in many (but not all) countries, only metformin (introduced in Europe in 1957) survived and is—with respect to tons of drug consumed—the world leader. For example, the influent concentration in 5 German wastewater treatment plants varied from 18 to 105 μg/L,[14] which is equivalent to an estimated metformin consumption in Germany of 370–897 tons per year. Metformin is an environmental contaminant, ends up in some plants, can be even highly enriched in seeds[15] and may be present in concentration of 1 to 3 μg/L in our drinking water. As a caloric restriction mimetic metformin increases life span in some laboratory animals and the worm *C. elegans*.[16] It is gaining experimental popularity as rejuvenating drug or gerosuppressant, being able to stimulate neurogenesis.[17] It acts as an antidote since it completely prevents gentamycin kidney toxicity in rats.[18] Highly significant in the context of this article, metformin ameliorates hepatic methotrexate toxicity in experimental animals.[19] Of equal interest for dermatology, metformin as a topical (or systemic) treatment blocks UV-B induced tumor formation in hairless mice[20] and reduces growth of squamous cell carcinoma.[21] As an adjunct to conventional cancer therapy or to prevent relapse more than 40 human trials with metformin are listed in ClinicalTrials.gov (www.clinicaltrials.gov accessed November 7, 2012). There is, however, an ongoing debate, if metformin acts via a systemic effect solely by decreased insulin levels (and/or less obesity) or has direct actions on tumor cells as is observed experimentally.[22] A detailed discussion of metformin and related biguanidines for cancer prevention and treatment is beyond the scope of this article and the reader is referred to excellent reviews (see refs. 148 and 149).

Metformin was first used for women with (and later without) diabetes to increase insulin sensitivity, promote ovulation and decrease androgen production from the ovaries, by lowering the "hyperinsulinemic" status (see ref. 23). However, adrenal cells in vitro are highly sensitive to metformin,[24] suggesting that subtle alterations of adrenal steroid metabolism may occur by direct action. Adrenal glands and especially the cortex due to the presence of transporters accumulate the drug similar to small intestine, liver, skeletal muscle and kidney.[25] As will be outlined later metformin appears to have some beneficial effects only in males, which may be related to differential effects on steroid hormones.

Metformin's status to improve fertility in PCOS has not been convincingly demonstrated,[23] but it certainly ameliorates symptoms of this highly prevalent condition. In the context of co-morbidities for psoriasis it is remarkable that PCOS women also have an increased risk of cardiovascular events and diabetes.[26]

Methotrexate: From an anti-neoplastic (anti-metabolic) to anti-inflammatory, vasculoprotective and "antidiabetic" drug. Methotrexate and its more toxic precursor aminopterin were first introduced as anti-neoplastic agents. More by chance the anti-folates, especially low-dose, once weekly methotrexate, found entry into dermatology, not only for treatment of psoriasis but for numerous other skin conditions.[27] Methotrexate is still the most effective disease modifying antirheumatic drug for rheumatoid arthritis and related disorders. More recently, based on data from observational studies with low-dose methotrexate for rheumatoid arthritis or psoriasis indicating lower cardiovascular risk,[28] it was suggested to employ methotrexate as "vasculoprotective drug" for patients with stable cardiovascular disease and elevated hsCRP.[29] Our concept, namely that part of the action of methotrexate is based on activation of AMPK via AICAR (ZMP), is supported by experiments with genetically diabetic (db^+/db^+) mice, where glucose and insulin levels are significantly lowered after only 4 weekly i.p. methotrexate doses (0.5 mg/kg).[30] Unfortunately, the authors failed to demonstrate that either AMPK or own of its downstream effectors such as acetyl-Coenzyme A -carboxylase were phosphorylated.

Metformin and methotrexate have some common features. *Uptake via carriers or transporters, elimination via the kidney and "deep compartement".* Both drugs are taken up into cells (including enterocytes of the intestine) by specific carrier/transporter systems. Methotrexate enters as a "blind passenger" via folate uptake mechanisms and is trapped intracellularly by polyglutamination.[27] Metformin is taken up via organic cation- (OCTs)[31] and plasma membrane monamine transporters.[32] Secretion of metformin by kidney tubules and liver luminal membranes is driven by multidrug and toxin extrusion (MATE) proteins.[33] Much is known about the pharmacogenetics of metformin especially for naturally occurring variants of OCT1 (import) and OCT2 (export by the kidney) but less so for methotrexate. For both drugs, systemic bioavailability after oral administration is dose-dependent and limited in capacity: the higher the dose the less percentage of it is adsorbed. Both are (mainly) excreted via the kidney albeit via different transporters. Therefore plasma clearance is depending on kidney function. Under conditions of weekly (MTX) or daily dosing (metformin) a "deep" compartement is building up. Higher polyglutamated (MTX-PGs) species slowly increase, if red blood cell MTX-PG content is taken as proxy for tissue (or target cell, "site of action") residence. A steady-state with respect to the composition of red blood cell MTX-PGs is only reached after several months,[34] often paralleling clinical improvement.[35] Although on a different time scale (hours and days), the distribution volume for metformin increases from about 1.6 L/kg after a single dose[36] to about 4 L/kg[25] under continuous dosing. This indicates a large tissue reservoir, mainly but not only dominated by expression and activity of the various transporters. Similar to MTX (and the MTX-PGs), metformin accumulates in red blood cells[37] reflecting "deep compartements" elsewhere. Existence of such compartements is also apparent by late phases in plasma or urine elimination kinetics after long-term daily dosing.[38] Metformin has a pK of 11.5 and is highly water soluble. According to first principles in pharmacology, as a singly positively charged molecule at almost all pH values in the body, it prefers to reside in lower pH in preference to higher pH. Therefore metformin can also distribute via simple diffusion but on a much prolonged time-scale in contrast to rapid transport via OCTs. The average metformin concentrations at steady-state and daily doses of 2–3 g in plasma of humans are around 1 mg/L (8 micromolar) with an upper limit of 2.4 mg/L.[25] After oral intake peak levels in portal blood exposing circulating blood cells and, later, hepatocytes are likely to be much higher.[39] Within cells 1000-fold higher concentrations compared with the cytosol are calculated to exist in the intermembrane space of respiring, phosphorylating mitochondria with a high membrane potential.[40] Similar arguments may hold for lysosomes, endosomes, caveolae, Golgi and other acidified organelles. Surprisingly little attention has been paid to the fact that the transporters involved in uptake and extrusion of metformin were not designed by nature for drugs but mainly for endogenous substrates. The Km values for metformin are ~1 mM for OCT1[41] and 0.24–2 mM for MATE1 and MATE2, respectively.[33] Considering that experiments with cells are often performed with 1 or 10 mM metformin for many (24–48–72) h (see refs. 42–44), one wonders if metformin as a competing substrate did not change import and export of endogenous metabolites or nutrients. One metabolite which is substrate for both importers and exporters is the essential amino acid tryptophan. The Km values for this amino acid for OCT1 or OCT2 are in the 5–10 mM range ~ten times higher than for metformin. Plasma concentrations of tryptophan are ~60 μM[45] and those for metformin are up to 20–30 μM, making competition very likely. Indeed, upon chronic dosing with metformin plasma or serum levels of tryptophan in patients increase.[46,47] Tryptophan concentrations in urine have recently proposed as markers to predict patients pharmacokinetic parameters for metformin determined by the naturally occurring human variants of OCT2.[48] Thus, it cannot be excluded that part of the metabolic and gene expression changes observed after organ perfusion, cellular experiments and in patients are a consequence of substrate competition with transporters. It is interesting to note that competition of the hypoglycemic biguanidines with mitochondrial (not plasma membrane) cation transporters was suggested as early as 1979.[42]

The mechanism by which methotrexate and metformin act is still not fully understood. *Methotrexate: Are adenosine, AICAR or reactive oxygen species (ROS) mediators of the anti-inflammatory activity?* In **Figure 1** we show a highly simplified version of the mechanism by which methotrexate is proposed to act as an "anti-inflammatory agent,"[27,43] namely by increasing adenosine efflux. Enhanced urinary secretion of AICA (5- aminoimidazole-4-carboxamide), a degradation product of AICAR and of adenosine, was first observed in patients with psoriasis treated with methotrexate,[44] pointing to an inhibitory action of MTX (or MTX-PGs) at the level of AICAR transformylase (AICART). The importance of adenosine as an anti-inflammatory mediator was

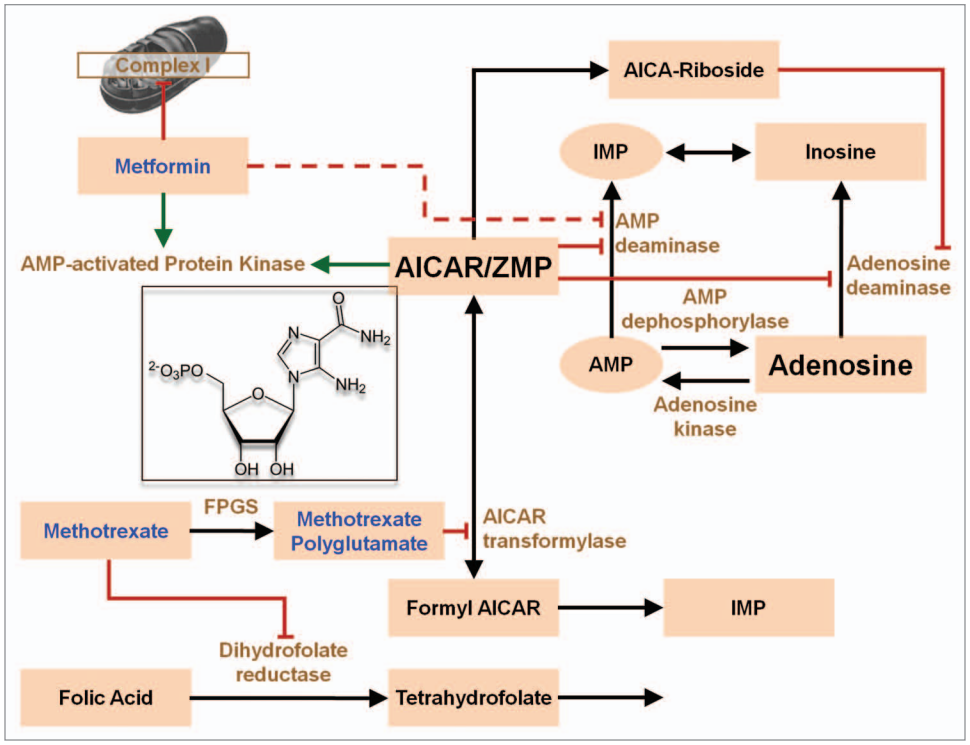

Figure 1. Metformin and methotrexate may interact at the cellular level via AMP-activated protein kinase. Simplified scheme[43,49] of the proposed "anti-inflammatory" activity of methotrexate. Increase of AICAR (structure shown in the inset) by inhibition of the bifunctional enzyme AICAR transformylase (AICART or ATIC)[50] is suggested to inhibit AMP and adenosine deaminase, leading to increased release of adenosine. Although it is generally postulated that the inhibition of AICART is by direct action of methotrexate polyglutamates, there are alternative explanations. These are based on the reversibility of the reaction (indicated by the double-headed arrow) and that AICART has rather low affinity for the polyglutamates. As an exception to all other reactions in mammalian one-carbon metabolism[51] 10-formyl-7,8-dihydrofolate and dihydrofolate can be substrate and product, respectively.[52] Increase of the product by blockade of the dihydrofolate reductase will slow the reaction and lead to increase in AICAR. FPGS, Folyl-Poly-Glutamyl-Synthase. Green arrows indicate activation; red lines with masthead indicate inhibition, broken line: disputed activity.

questioned[53,54] and could not been convincingly demonstrated in later experiments.[55] It is, however, generally accepted that AICAR (ZMP) increases after methotrexate[56]—either by direct inhibition of AICART or alternatively, favoring the reverse reaction by increasing dihydrofolate via blockade of dihydrofolate reductase.[52] (For more details, see the legend of **Fig. 1**). Another inhibitor of thymidylate synthase and AICART, pemetrexed, increases ZMP and activates AMPK.[57-59] In summary, experimental evidence in cell culture, animal experiments[30] and observations in patients support the view that methotrexate and related compounds can increase AICAR production and activate AMPK in addition to their inhibitory effects on nucleotide synthesis, which is still the claimed basis for immune-suppressive activity. Nevertheless, the molecular mechanisms underlying the "anti-inflammatory" activity and the postulated targets cells (T-lymphocytes in psoriasis, macrophages and monocytes in rheumatoid arthritis) are still not completely understood. The increased AICAR production and appearance of its degradation product AICA in the general circulation after methotrexate are intriguing. Does the release indicate rapid apoptosis of target cells or a continuous excretory mechanism? In any event, AICA and perhaps the corresponding riboside could act as a kind of "second messenger" to neighboring cells or elsewhere. The riboside may be taken up by the ubiquitous adenosine transporter and phosphorylated by adenosine kinase to ZMP. The purine base AICA may be taken up by nucleobase transporters[60] and converted via adenine-phosphoribosyl-transferase to ZMP. That AICA (and the riboside) could be the mediators or signals for clinical efficacy was already suggested by.[55] Reactive oxygen species (ROS) as mediators for methotrexate were postulated,[54,61] but this was not supported by subsequent research.

AICAr is often employed as an experimental tool in animal models for disease (see **Table 1**) or given i.v. in human studies (as acadesine)[63,64] to activate AMPK as an "AMP mimetic." Despite the expectation that the increased glucose uptake of human skeletal muscle after AICAr infusion was mediated by AMPK activation no increased phosphorylation of the master kinase was observed. Instead the extracellular signal-regulated kinase 1/2 (ERK1/2) was phosphorylated.[65] A word of caution is appropriate here, as some of the experiments claiming AMPK activation as a result of AICAr treatment fail to rigorously prove the claim as direct activators (A-769662) have differential effects on pyrimidine metabolism and the purine salvage pathway compared with AICAr.[66] Notably, however, is that AICAr can influence human glucose metabolism in vivo, underlining the hypothesis that it can serve as a signal[55] not necessarily via AMPK.

Metformin: A drug in search of a primary target. *Mitochondrial complex I inhibition, the mitochondrial permeability pore, the*

Table 1. Metformin and AICar exhibit anti-inflammatory and immunosuppressive activity in experimental systems

Experimental system	Outcome/results	Reference
Induced autoimmune encephalomyelitis (EAE) in female mice	Metformin (100 mg/kg body weight per day either i.p. or orally in 3 doses) improved disease score; inhibits immune cell infiltration into CNS, lowered increased levels of IL-17, IL-1β, IFNγ, TNFα and raised AMPK	112
Macrophage cell line Primary Macrophages	Metformin and AICAr block LPS-induced secretion of TNFα, IL-6 and IFNγ. AMPK is activated	112
Naïve CD 3-positive T cells	Metformin (1–10 mM) inhibits T-cell proliferation and IFNγ, IL-17 secretion upon stimulation	112
Mouse model for acute and relapsing colitis induced by 2,4,6 trinitrobenzene (TNBS)	AICAr (500 mg/kg body weight daily i.p.) attenuates weight loss and improves colon inflammation. Levels of TNFα, IFNγ and IL-17 are significantly lowered in colon homogenates. AICAr increased pAMPK (Thr 172), decreased iNOS and elevated pNFK B p65.	113
Mouse model for chronic asthma induced by immunization with ovalbumin and fungal associated allergenic protease	Metformin (250 mg/kg body weight) or AICAr (100 mg/kg) applied 30 min before each intranasal challenge twice a week decreased airway inflammation score, lymphocyte and eosinophile cell counts in bronchoalveolar lavage. Metformin (but not AICAr) decreased ovalbumin-specific IgG 1.	114
Experimental autoimmune uveitis in female mice, induced by human interphotoreceptor retinoid binding protein	AICAr (200 mg/kg body weight, injected daily i.p.) reduced severity of EAU (clinically and in histopathology, even 8 d after immunization)	147

metformin-copper hypothesis and phosphatase inhibition. Ever since guanidines were reported to inhibit energy transfer reactions of mitochondria in 1963,[67] interest has focused on their effects mitochondrial function, including transport of divalent cations,[68] the still unexplained reversal of respiratory inhibition by uncouplers or fatty acids[69] and much more. Accepted today as a prime (not primary!) target is complex I. Complex I (NADH-ubichinone-reductase) is part of the respiratory chain located in the inner mitochondrial membrane. This huge multi-subunit complex (around 950 kDa) pumps four protons out of the matrix into the inter-membrane space tightly coupled to the reduction of ubichinone and oxidation of NADH $+H^+$. Metformin has inhibitory action (only) on complex I of mitochondria[40,70] and can thereby change the cellular ATP production via oxidative phosphorylation. The mystery is that the inhibitory effect (which is never higher than about 40% of the maximal activity) can only be observed when metformin acts on "intact" isolated cells (permeabilized cells are unresponsive) or after organ perfusion with the drug. Mitochondria isolated from such pretreated cells appear irreversibly impregnated, as if metformin somehow, passing plasma cell membranes, acquired a novel property which cannot be mimicked by mixing cytosol and/or plasma membrane particles in the presence of metformin with naive mitochondria. This has led one group of the original discoverers to the suggestion that metformin somehow changed the mitochondrial machinery via phosphorylation/ dephosphorylation or proteolysis.[70] One argument was that the degree of inhibition of complex I in isolated intact hepatocytes but not its kinetics was a clear function of the temperature, being optimal at 37°C and almost absent at 15°C. However, it was not known at the time that transport of metformin via OCT1 is highly temperature dependent.[41] In any event, inhibition of complex I and the resulting changes of the AMP/ATP ratios are believed to be responsible for the activation of AMPK[71] (see below). It should be noted that in contrast to AMPK activation, demonstrated in humans after chronic dosing with metformin, complex I inhibition of isolated mitochondria from skeletal muscle biopsies was not observed.[72]

Inhibition of complex I by metformin (similar and additive to blockade of cyclophylin D via cyclosporine A) sensitizes the permeability transition pore (PTP) in the inner mitochondrial membrane to attain a state of lower open probability.[73,74] The pore plays an important role in cell death induced by free radicals, UV radiation, oxidative stress or ischemia. Results with metformin as a "protecting agent"[18,20] suggest that there may be benefits of the drug beyond AMPK activation as explained in the following paragraph.

Inhibition of complex I of mitochondria isolated from cells pretreated with metformin leads to a block in "reverse electron flux"-induced superoxide anion formation.[75] Superoxide anions may be formed by escaping electrons at complex I and III. When there is, in simple words, some kind of block downstream of complex I, electrons can flow back, reduce NAD to NADH, increase the ratio of ubiquinol to ubiquinone and contribute to superoxide formation. Superoxide anion cannot pass the inner mitochondrial membrane and is converted to hydrogen peroxide by manganese superoxide dismutase. In the cytosol H_2O_2 can activate redox-dependent transcription factors and is an indispensable signal (as is calcium) for T-cell activation.[76,77] Thus, metformin by reducing ROS, can profoundly change T-cell responses in experimental systems.

Metformin and related biguanidines are excellent chelators (e.g., zinc and copper).[78,79] Metformin can form stable complexes[80] with copper (association constant around 10^{16} M^{-1}). A more apolar (hydrophobic) dimeric metformin Cu^{2+} complex, which must pass the plasma membrane, slowly forms inside cells and accumulates in mitochondria is now suggested to be the key mediator.[81,82] If so, metformin could be a "prodrug," but the final activity may be not via simple copper chelation.

Other biguanido metal complexes are very potent inhibitors of protein phosphatases[83] suggesting that copper or other metal

proteins could be among the long sought "primary" targets. Metformin can inhibit tyrosine phosphatases (such as recombinant human protein phosphatase 1 B) in vitro at very low concentrations (8 μM) but only if plasma membrane fragments (from oocytes) are added.[84] One wonders about this truly magic potentiation, but perhaps, formation of an active "intermediate" with a phospholipid, a metal ion or a metallo protein occurred.

AMPK activation. Metformin activates a master regulator in metabolism, the AMPK,[77,85] via increased phosphorylation of its catalytic α-subunit in position threonine-172, mediated by LKB1. This drug-induced activation is proven by animal experiments,[86] in experimental cell systems[71] as well as in humans after chronic dosage.[87,88] AMPK consists of three subunits where the regulatory gamma subunit is functioning as a cellular AMP or ADP sensor, which changes its conformation upon binding AMP or ZMP or other activators such as salicylate[89] and increases the availability of the α subunit to be substrate of upstream protein kinases. Metformin binds to the gamma subunit of AMPK in vitro[90] but in contrast to direct activators such as A-769662,[91] salicylate, ZMP, AMP or OSU-53[92] activation of the enzyme in cell free systems has never been reported. Metformin cannot activate AMPK in cells where the gamma subunit of the enzyme is manipulated to be unresponsive to AMP. Consequently it is generally accepted[71] that by lowering the cellular energy status and changing the ATP /AMP ratio via mitochondrial inhibition AMPK is activated. An alternative view, namely that metformin acts by inhibiting AMP deaminase[93] is questioned.[85] It is not necessary here to discuss the many downstream targets of AMPK, its connections to the mTOR signaling pathway[94] or its role in the regulation of sirtuins.[95] We also omitted that increased intracellular calcium (via calmodulin-dependent protein kinase β) can phosphorylate and activate AMPK, as no evidence so far was found that metformin acts through this pathway.

The physiological role of the AMPK is mainly to control metabolism, depending on nutrient supply. Thus it is natural to assume its main action in diabetics is on liver, adipose tissue and skeletal muscle. However, there is good evidence that this master protein kinase is also involved in the regulation of inflammatory responses and has controlling activity in dendritic cells, T-lymphocytes, macrophages, endothelial cells and monocytes. This is no surprise, as often these cells must either rapidly proliferate (and switch to more glycolysis[96,97]) or synthesize, e.g., cytokines for secretion and other mediators, which require major changes in metabolism and import of nutrients.

AMPK independent activities of metformin. Inhibition of mTORC1 (MammalianTarget of Rapamycin Complex I) or activation of protein phosphatase 2A by metformin (and phenformin) were shown to be independent from AMPK,[98-100] as well as most of the stimulation of glucose uptake in myotubes.[101]

Is metformin a methotrexate mimetic? Methotrexate increases plasma homocysteine levels in patients with psoriasis.[102,103] Metformin (upon long-term treatment) can lower plasma vitamin B_{12}[104] and increases plasma homocysteine levels.[105,106] The underlying assumption is that metformin impairs the intestinal reabsorption of vitamin B_{12}. However, short-term studies in diabetic patients,[107] non-diabetic males[108] and patients with PCOS[109] indicate that the increase in homocysteine levels is not correlated with vitamin B_{12} deficiency. Plasma levels of the most sensitive indicator of vitamin B_{12} deficiency, methymalonic acid, are not changed. Furthermore, the increase in homocysteine can be completely prevented or corrected by administration of folate and conventional doses of oral vitamin B_{12} are unable to attain "normal levels" in metformin treated patients.[104] The reversal by folate (and not by vitamin B_{12}) indicates that metformin inhibits target(s) in tetrahydrofolate-dependent metabolic pathways. These observations stimulated a study in which metformin was employed at rather high concentrations in several breast cancer cell lines. Here it was found, by analysis of metabolic changes, that metformin dramatically increased the intracellular levels of 5-formiminotetrahydrofolate.[110] This metabolite is the product of histidine catabolism to glutamate and can be converted by two subsequent enzymatic steps to N,^{5} N^{10}-methylene-tetrahydrofolate. This is the essential substrate (not cofactor) for thymidilate synthase. The authors did not speculate how metformin acts as an "antifolate" but as expected from the ability of metformin to block transport of endogenous substrates (see the section "Uptake via carriers or transporters, elimination via the kidney and "deep compartement") they observed a marked decrease in intracellular tryptophan, which delivers formate to tetrahydrofolate in the pathway to kynurenines and is precursor for NAD^+. Metformin also decreased glutathione which was confirmed by others.[111]

Consult **Table 2** for a brief summary of the accepted targets for metformin and methotrexate.

Metformin is an anti-inflammatory drug. *Evidence that metformin or/and AMPK activation via AICAr exhibit anti-inflammatory and immunosuppressive activity in cellular and animal experiments.* In **Table 1**, we present key publications that support the view that metformin (and AICAr as an AMP-mimetic) is anti-inflammatory and immunosuppressive in various experimental mouse models of autoimmune diseases. The key role of AMPK in macrophages was demonstrated by Sag et al.[115] They observed that anti-inflammatory stimuli such as IL-10 or TGFβ increase levels of phosphorylated AMPK within minutes, whereas stimulation of Toll-like receptors (TLR4) by lipopolysacccaride (LPS) decreases AMPK activation. TNFα, IL-6 secretion after LPS was increased by stably transfecting with a dominant negative AMPK but IL-10 decreased. Exactly opposite changes were observed by transfection with a constitutively active AMPK. In dendritic cells the anti-inflammatory cytokine IL-10 partially blocked the hypophosphorylation of AMPK by LPS and AICAr blocked the LPS induced maturation.[116] The role of AMPK in T-lymphocytes is reviewed by ref. 117. All human white blood cells (neutrophils > monocytes > lymphocytes) express OCT-1 and can transport metformin.[118] Human monocytes in vitro are highly sensitive to metformin. Concentrations observed in patients (10 mM) blocked tissue factor activity increase and TNF release after exposing cells to oxidized LDL or LPS.[119] T-lymphocytes play a key role in psoriasis and other inflammatory processes therefore inhibition of complex I by metformin[77] and reduced ROS formation are possible beneficial actions independent from AMPK.

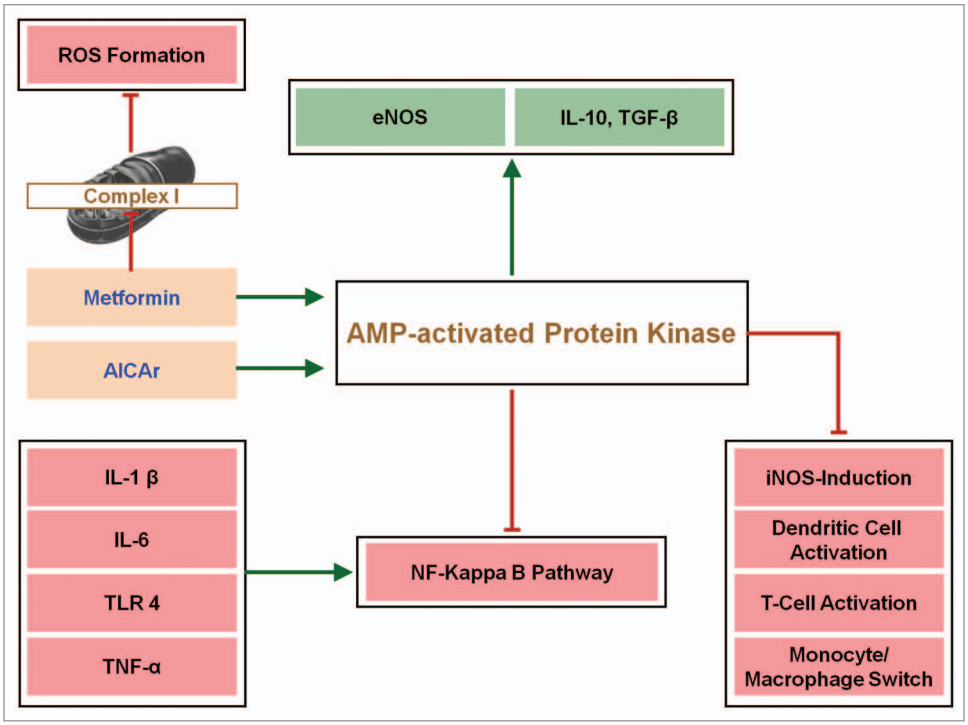

Figure 2. Simplified scheme how metformin and AICAr act as anti-inflammatory or immunosuppressive agents. Green arrows indicate activation or stimulation; Red lines with masthead indicate inhibition. Metformin may not only stimulate AMPK but block excessive reactive oxygen species (ROS) formation by mitochondria, which can activate the Nuclear Factor (NF)-KB pathway (not shown). For details on the role of AMPK in macrophages, see ref. 115. Molecular detailed pathways are found in ref. 120.

In **Figure 2**, we show a highly simplified scheme, how metformin and AICAr may exert anti-inflammatory and immunosuppressive activity (For more molecular details, see ref. 121).

Evidence that metformin decreases inflammatory markers in patients. In the first section of our *Results*, we mentioned briefly that patients with rheumatoid arthritis were successfully treated with phenformin. The intervention was based on the hypothesis that phenformin acts as a fibrinolytic drug. Indeed clinical symptoms of inflammation improved in some patients and the elevated erythrocyte sedimentation rate (ESR), substituted now by CRP, fell significantly. The same authors treated 27 patients with coronary-artery disease for four months with 1,500 mg metformin/day, followed by two months placebo. Twenty-one patients (there were seven drop-outs) demonstrated an increase of plasminogen activator and lower cholesterol. They also noted that the effect was greatest in patients with lowest fibrinolytic activity and that upon cessation of the drug it took a month or more until the starting level was reached.[122] Significant decreases of tissue-type plasminogen activator inhibitor and of von Willebrand factor were later confirmed in a randomized double-blind controlled trial with obese subjects but no overt diabetes.[121] Other symptoms of the metabolic syndrome, such as higher systolic blood pressure and elevated LDL-cholesterol also improved.[123] In non-obese patients with type 2 diabetes, comparing metformin with repaglinide, markers of endothelial dysfunction (plasma plasminogen activator inhibitor-1 antigen, von Willebrand factor) and TNFα were significantly decreased by metformin.[124] A recent placebo-controlled study in which subjects with impaired fasting glucose (and other symptoms of metabolic syndrome) were treated for 3 mo with simvastatin received metformin as "add-on" experimental treatment. Here it was observed that even in the background of statin-induced "anti-inflammatory" activity, metformin inhibited lymphocyte cytokine release (TNF α, Interferon γ, Interleukin 2).[117] There are a few studies with metformin in patients with PCOS. A randomized comparative study proves a significant decrease of IL-6,[125] which is confirmed by observations in Taiwan.[126] Others found significant decreases of hsCRP[127,128] after metformin. With the possible exception of the early discovery of the anti-inflammatory and anti-fibrinolytic activities of the biguanides, all other studies with metformin were performed in patients or subjects with elevated basal insulin levels or increased insulin resistance. The generally accepted interpretation is that the decrease of inflammatory markers is mediated by increased insulin sensitivity or lowering of insulin levels in PCOS and weight loss. However, the animal models listed in **Table 1** argue that anti-inflammatory effects of metformin are observable under experimental conditions where insulin and/or obesity are not drivers of inflammation.

Psoriasis and metabolic syndrome. Inflammatory signaling mechanisms play an important role in psoriasis[129] and may contribute for developing so-called co-morbidities, such as higher cardiovascular risk or type 2 diabetes.[130] Indeed—depending on severity—psoriasis is associated with metabolic syndrome[131-133] and diabetes.[134,135] Cohort studies prove an association of psoriasis with increased, mainly cardiovascular mortality.[136] Interrogation of the psoriasis transcriptome in comparison to healthy controls

Table 2. Molecular mechanisms and targets for metformin and methotrexate

Whereas for most of the drugs commonly used "primary targets" (e.g., drug receptors) responsible for therapeutics can be identified,[62] no such primary target is identified for metformin. It is therefore doubtful if drug agencies would accept metformin today, as a defined target is often conditional for approval.
Possible primary targets for metformin are the transporters. It may compete with the in-and efflux or excretion of endogenous substrates or nutrients and other drugs. If this results in so-called "off-target" effects, or is of no consequence or even contributes to therapeutic efficacy is yet not known.
Established "secondary" targets of metformin, important for therapeutics, are mitochondrial complex I (inhibition), also believed to contribute to lactic acidosis after toxic doses/plasma concentrations and AMPK (activation).
Accepted "primary targets" of methotrexate and its polyglutamates are tetrahydrofolate-dependent enzymes in nucleotide biosynthesis. A secondary target may be AMPK (activation) via increase of AICAR.

revealed links to pathways involved in atherosclerosis, fatty acid metabolism, diabetes and hypertension.[137] A recent meta-analysis including all results from this and other studies, employing ingenuity pathway analysis, found overrepresented canonical pathways for atherosclerosis, cancer and cardiovascular disease. Interestingly, pathways identified in the pathogenesis of multiple sclerosis (see **Table 1**) were also among the top five.[138] Needless to say, that the same pathways are in the focus of current research with metformin.

Expanded intra-abdominal adipose tissue is suggested to play an important pro-inflammatory role in obese patients with metabolic syndrome and psoriasis. Correcting extreme obesity with bariatric surgery indeed improves psoriasis,[139] and one study suggests that weight loss per se had an additive effect to improve PASI scores.[140] As will be discussed below metformin is able to prevent progression of patients with metabolic syndrome to overt diabetes and can induce significant and permanent weight loss solely due to reduction of adipose tissue.

Metformin is the only drug with a favorable benefit/risk profile to prevent progression to type 2 diabetes for patients with metabolic syndrome and/or reduced glucose tolerance. Although glitazones and α-glucosidase inhibitors may prevent progression of prediabetes[141] or favor regression[142] to normoglycemia we will not discuss these drugs. Instead a few results of the metformin and lifestyle intervention study, DPP (diabetes prevention program)[143] and its open label follow-up study[144] are interesting in the context of our proposal. Metformin reduced incidence of metabolic syndrome and favored its regression but only in males.[145] The authors speculated that hormonal alterations are responsible for this highly unexpected result. Weight loss is highly correlated with adherence to metformin and solely due to loss of adipose tissue.[146]

Methods

We searched the literature via pubmed, Google Scholar, Web of Science with key phrases alone and (more) often in combination: biguanide(s), phenformin, metformin, AICAR, AICAr, methotrexate, T-lymphocytes, monocytes, macrophages, anti-inflammatory, metabolic syndrome, psoriasis, AMP-activated protein kinase, AMPK, reactive oxygen species, complex I (inhibitors), inflammation, inflammatory biomarkers, clinical trials, organic cation transporters. We also approached some of the leading researchers in the field to supply us with further information including publications in press.

Conclusion

Metformin is a logical add-on therapy for overweight male patients with psoriasis and metabolic syndrome treated with methotrexate. Several of the newly introduced, highly expensive drugs for psoriasis (e.g., anti-cytokines) are "targeted therapeutics." In contrast, although approved as an antidiabetic or employed to lower hyperinsulinemic conditions (PCOS) a primary target of the long off-patent and cheap metformin is not (yet) known. This probably explains complete lack of clinical observations not to mention human trials, based on its ability to act, among other, as a powerful anti-inflammatory and immunosuppressive drug. Without a doubt, metformin is—next to lifestyle intervention and for example, statins—a most logical choice for male overweight psoriatric patients with metabolic syndrome. This is ethically justified because evidence exists in the literature that it can prevent further detoriation of prediabetes or regress symptoms of metabolic syndrome.

We collected arguments to support the hypothesis that there may be additive anti-inflammatory effects of metformin for methotrexate-treated patients, which can be first tested in a prospective, open-label clinical pilot trial. Perhaps of equal importance, metformin decreases hepatotoxicity of methotrexate in animal experiments. This observation alone, taken up as a lead, could stimulate clinicians first to check retrospectively if metformin, once started, improved signs for hepatic injury after methotrexate.

Fifty years ago, researchers who treated patients with rheumatoid arthritis (successfully) and coronary artery disease with phenformin or metformin, respectively, could measure only a few laboratory parameters.

Today's diagnostic armentarium (including the OMICS world) is much greater and in addition to for example, the PASI score, biomarkers systemically and locally can be followed. Both drugs have the same limiting factor, namely kidney function. A major concern is abdominal discomfort after metformin,[20,*] which may be reduced by starting with low doses (e.g., 0.5 g/day) and retarded formulations, slowly escalating up to the tolerated limit of 2.5 g/day. As a further safety measure, a metformin drug pause is suggested for the day methotrexate is given. Of less importance is the mechanistically unexplained increase of serum or plasma homocysteine after metformin because patients routinely take folate.

* If abdominal discomfort is a problem and since dermatology is interested in transdermal delivery or local therapy, the reader is referred to www.freepatentsonline.com/y2012/0283332.html.

If our hypothesis is supported by favorable results in a pilot trial, perhaps one can expect less use of "targeted" drugs in the future. Even if our hypothesis of an additive anti-inflammatory effect is disproven, male, overweight patients with psoriasis and metabolic syndrome, given that metformin is tolerated, will profit from the intervention.

Disclosure of Potential Conflicts of Interest

Both authors have received an honorarium by Pfizer for their contributions at a Symposium in Vienna on occasion of the 50th anniversary of methotrexate. No other conflicts of interest exist with respect to the above work.

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