Antiatherogenic properties of metformin: the experimental evidence

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SUMMARY
Cardiovascular disease (CVD) is the major determining factor of morbidity and mortality in type 2 diabetic patients. The established relationship between type 2 diabetes and atherosclerosis has fuelled suggestions that anti-diabetic drugs with beneficial effects on CV risk factors may help attenuate the atherosclerotic process in diabetic patients. Metformin is a hypoglycaemic agent widely used in the management of type 2 diabetes. In addition to its insulin-sensitising action, this drug has favourable effects on various CV risk factors and reduces macrovascular complications in obese type 2 diabetic patients. This review summarises in vivo and in vitro experimental evidence on the antiatherogenic properties of metformin.

Key-words: Metformin · Type 2 diabetes · Atherosclerosis · Insulin resistance · Atheroprotective effects.

Cardiovascular disease (CVD) is the major determining factor of morbidity and mortality in type 2 diabetic patients [1], the risk of developing CVD in these subjects being 2 to 4 times higher than in non-diabetic subjects [2]. The pathogenesis of CVD is multifactorial and can be affected by metabolic and other factors, including insulin resistance, hyperglycaemia, hypertension, hyperlipidaemia, and alteration of the coagulation system [3-5]. The established relationship between type 2 diabetes and atherosclerosis has fuelled suggestions that anti-diabetic drugs with beneficial effects on CVD risk factors may help attenuate the atherosclerotic process in diabetic patients.

Metformin (N\(^1\), N\(^2\)-dimethylbiguanide) is an insulin-sensitiser, the predominant effect which is to reduce hepatic glucose production and to increase the sensitivity of liver and peripheral tissues to insulin [6, 7]. In the United Kingdom Prospective Diabetes Study (UKPDS), metformin monotherapy was found to be associated with reduced macrovascular complications and all-cause mortality in overweight patients with type 2 diabetes [8]. Furthermore, administration of this drug has recently been shown to be associated with decreased all-cause and CV risk of mortality [9]. Such improvements in the CV outcomes seen with metformin do not seem to be related to glycaemic control, but rather to specific vasculoprotective effects of this drug. This review summarizes experimental evidence on the antiatherogenic properties of metformin and outlines mechanisms by which this drug may affect atherosclerotic lesion development or progression.

Overview of animal models

Atherosclerosis is a progressive and multifactorial disease involving a number of genetic and environmental factors [10]. Direct human atherosclerosis-related research is limited by the silent and asymptomatic nature of the disease at its early stages, the slowness of lesion development and the inability to sequentially characterise lesions [11, 12]. Thus, since the turn of the last century, there has been a reliance on animal models of atherosclerosis to better define mechanisms involved in the development and the progression of the disease.

Animal models of atherosclerosis are expected to fulfill several requirements, including low cost, speed of breeding, and a well-defined genetic background [11, 13]. Importantly, the animal models should have some similarities to the human anatomy and physiology and share the pathophysiology of the disease with humans [11, 13, 14]. Unfortunately, the ideal animal model that completely reproduces human lesions does not exist [11, 13, 15]. Rabbits, one of the most investigated animals, have been found to develop medial fatty and macrophage-rich lesions that do not resemble human lesions [11, 16, 17]. These animals, however, have the advantage of developing aortic lesions in a short time on being fed cholesterol-enriched diets. Avian species, including the pigeon, the turkey and the Japanese quail, are not currently used in atherosclerosis research because of the small size of their arteries [18]. Rodents and dogs are not viewed as good models because they do not develop spontaneous lesions, unless fed a heavily modified diet to induce vascular lesion [11, 16]. Early lesions in inbred strains of mice such as the C57BR/cdJ are morphologically similar to human lesions, whereas advanced lesions are medial in location and inconsistent [19]. The pig and nonhuman primates, including monkeys, are good models because they develop spontaneous lesions with distribution, pathogenesis, and morphology similar to that of humans. Limitations of the pig model are the cost and the difficulty of handling, whereas monkeys are not widely used nowadays because of several concerns, including the cost and government regulations [11, 18].

Over the past few years, transgenic/knockout animal models have greatly advanced our understanding of the pathogenesis of atherosclerosis. In contrast to wild-type counterparts, these animals develop atherosclerosis faster without the need for a highly atherogenic diet [14]. The mouse is the primary transgenic model used in atherosclerosis research [20]. Studies using transgenic mice have allowed definition of the relationship between lipoprotein abnormalities and atherosclerosis. In addition, they have demonstrated that nonlipid factors such as oxidative stress, infection, and inflammation may influence the severity and characteristics of atherosclerotic lesions [12, 14]. The use of animals as models to evaluate the therapeutic potential of pharmaceutical drugs in humans is still a matter a debate. Researchers should be aware of the shortcomings of the experimental model used to better interpret data and develop therapeutic strategies for humans.

Effect of metformin on experimental atherosclerosis

The lesions of atherosclerosis are generally classified into three categories: the early lesion or fatty streak, the fibrous lesion, and the advanced or complicated lesion [21]. A primary initiating event in atherosclerosis is endothelial dysfunction (ED) which promotes vascular inflammation through several mechanisms, including increased adhesion of monocytes and enhanced endothelial permeability to monocytes and lipoproteins, leading to foam cell formation [22]. ED is closely linked to CV risk factors and evidence suggests that hyperglycaemia, hypertension and hyperlipidaemia induce ED through oxidative stress [23].

As early as in the late 1960s, several studies were conducted to examine the effect of metformin on experimentally-induced atherosclerosis. In those years, atherosclerosis research was largely focused on the relationship between plasma lipid metabolism and aortic lesion formation. Thus, researchers mostly investigated whether metformin may prevent atherosclerosis through its ability to alter plasma and aortic lipid metabolism in cholesterol-fed animals. More recently, understanding of the molecular and cellular mechanisms of
Atherosclerosis has prompted considerable interest in assessing whether metformin may inhibit vascular cell dysfunction induced by atherosclerosis-promoting agents. Several observations suggest that metformin may inhibit the level of plaque formation in animal models of atherosclerosis. A preventive effect of metformin on aortic lesion development was first reported in cholesterol-fed male Fawn Bourgogne rabbits [24]. Subsequent studies in animals such as the New Zealand rabbits fed a hyperlipidaemic or cholesterol-enriched diets [25-27, 28, 29] and the Wistar rats [30] further demonstrated an inhibitory effect of metformin on plaque formation. In most of the studies, the beneficial effect of metformin on arterial lesion formation was not related to the level of plasma lipids, thus suggesting a vascular effect of the drug. Morphological observations have shown that metformin prevents endothelial lesions and damage to the elastic framework of the aortic wall caused by cholesterol feeding [26, 28]. Several mechanisms may account for the inhibitory effect of metformin on aortic lesion development. Among these, a suppressive effect of this drug on cholesterol ester deposition in the aorta and an alteration of arterial lipid metabolism have been proposed. Supporting this possibility, treatment of cholesterol-fed rabbits with metformin has been found to markedly decrease the synthesis of all major lipid fractions in cholesterol-fed New Zealand rabbits, where administration of this drug signifi-
cantly decreased cholesterol ester accumulation in the aorta, causing thereby regression of atherosclerosis [32].

Effect of metformin on atherosclerotic lesion initiation and development

Enhanced monocyte adhesion to the vascular endothe-
lum together with increased infiltration of lipoproteins in the arterial wall represent the first and determinant phase of atherogenesis [21]. Once in the arterial wall, newly recruited monocytes undergo activation-differentiation and transformation into macrophages which secrete proatherogenic fac-
tors [33]. Mechanisms responsible for monocyte binding involve the upregulation of leukocyte and endothelial cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin [34]. The expression of these adhesion mole-
cules is regulated by various stimuli including high glucose concentrations, oxidised low-density lipoproteins (oxLDL), and advanced glycation end products (AGEs) [35, 36]. Recently, the effect of metformin on leukocyte-endothelial cell interaction has been investigated in vitro. It has been shown that incubation of cultured endothelial cells with metformin inhibits AGE-induced monocyte adhesion to these cells as well as endothelial cell surface expression of ICAM-1, VCAM-1, and E-selectin [37]. This later effect probably accounts for the suppressive effect of metformin on monocyte binding to endothelium. Previous results demonstrating that metformin decreases leukocyte adhesion to microvascular cells in vivo [38] suggest that the observed in vitro effect of this drug may translate into a decreased monocyte adhesion to endothelium in vivo. Interestingly, metformin also appears to inhibit differentiation of human monocytes into macrophages in vitro (Fig 1) [37]. Although the molecular mechanisms underlying this effect remain unknown, it is possible that metformin may interfere with mechanisms governing monocyte differentiation into macrophages, including expression of integrin genes and activation of various transcription factors [39, 40]. Taken together, these in vitro observations suggest that metformin treatment may inhibit early biological events involved in the formation of atherosclerotic lesions.

A number of studies have also investigated the effect of metformin on arterial lipid metabolism. In normal Fawn Bourgogne rabbits, administration of metformin was found to decrease aortic cholesterol ester accumulation as assessed by labelled acetate incorporation into arterial lipids [41]. A similar observation was made in cholesterol-fed New Zealand rabbits, where administration of this drug significantly decreased cholesterol ester accumulation in the aorta, without affecting plasma cholesterol [25-27]. Study of the composition of very low density lipoproteins (VLDL) in cholesterol-fed New Zealand rabbits demonstrated that metformin has beneficial effects on the chemical composition of these diet-induced atherogenic particles, increasing their phospholipid and triglyceride content, while decreasing their sphingomyelin content [25]. Investigation of the turnover and aortic uptake of VLDL from hypercholesterolaemic rabbits showed that administration of metformin accelerates catabolism of VLDL and induces an efficient conversion of these particles to lipoproteins of higher density [42]. Compared to VLDL from untreated cholesterol-fed animals, VLDL from metformin-treated rabbits further exhibited decreased binding affinity for the arterial wall [42]. Because lipoprotein trapping in the arterial wall is central to lesion development [43], decreased arterial retention of atherogenic lipoprotein in metformin-treated animals may represent a protective effect of this drug in the earliest stages of atherogenesis.

Figure 1
Metformin inhibits the process of monocyte differentiation into macrophages. Freshly isolated human monocytes were cultured for 9 days at 37°C in the presence or absence of metformin. 1: control cells; 2: metformin 0.5 µg/ml; 3: metformin 1 µg/ml. Representative phase-contrast photomicrographs from 3 independent experiments are shown (magnification x 100).
In the presence of modified lipoproteins, macrophages present in the lesion convert into foam cells [21]. Recently, it has been demonstrated that metformin inhibits foam cell formation induced by minimally modified LDL in vitro (Fig 2) [37]. Evidence that metformin decreases the expression of receptors involved in cellular lipid uptake, including scavenger receptor A and lectin-like oxidised LDL receptor [37], suggest that this drug exerts its effect through its inhibitory effect on these cell surface receptors. These results may provide a rational explanation for previous data reporting an inhibitory effect of metformin on intracellular lipid accumulation in the aorta of rabbits fed a hyperlipidaemic diet [27, 28].

As fatty streaks progress to fibrotic lesions, smooth muscle cells migrate from the media to the intima, proliferate and become the predominant cell type of the lesion. Smooth muscle cells are responsible for the synthesis of factors such as extracellular matrix components and growth factors that contribute to lesion progression [44]. Administration of metformin to cholesterol-fed rabbits has been shown to inhibit smooth muscle cell proliferation [26], an effect that may account for the beneficial effect of this drug on aortic lesion formation reported in that study. In vitro, metformin has been shown to inhibit vascular smooth muscle cell proliferation in response to platelet-derived growth-factor (PDGF) by reducing agonist-stimulated intracellular calcium concentration [45].

**Effect of metformin on aortic lesion complication**

The advanced or complicated lesion of atherosclerosis results from necrosis, calcification, and mural thrombosis of fibrous plaques. Although there are currently no reports on the effect of metformin on aortic lesion complications in animal models of atherosclerosis, a compelling body of data suggests that metformin may possess adjunctive properties that may prevent the formation of complicated lesions. Indeed, evidence has been provided that metformin inhibits thrombus formation in a carotid occlusion model [46] and in laser injured arteries [47]. Furthermore, results from in vitro studies suggest that this drug may increase total fibrinolytic activity by reducing plasma levels of plasminogen activator inhibitor-1 (PAI-1) [48]. Recently, it has also been demonstrated that metformin decreases thrombin-induced fibrinopeptide cleavage from fibrinogen as well as fibre thickness and pore size of fibrin clots, thus suggesting that the cardioprotective effect of this drug may be related to effects on clot stabilisation [49]. Finally, it has been shown that administration of metformin to hypercholesterolaemic rabbits decreased platelet aggregation, a cellular event associated with increased CV risk in type 2 diabetes [50].

**Effect of metformin on arterial compliance in animal models**

Atherosclerosis induces functional and structural changes that might influence arterial compliance. Although there is a paucity of information on the effect of metformin on arterial compliance in animal models of atherosclerosis, a beneficial effect of this drug on arterial compliance has been reported in hypertensive and diabetic animals [51, 52]. Because hypertension and diabetes increase the risk of developing atherosclerotic vascular complications, one may suggest that the reported beneficial effect of metformin may apply to the atherosclerotic disease.

![Figure 2](image-url)

**Figure 2**

Metformin (MET) inhibits foam cell formation induced by minimally modified LDL. Freshly isolated human monocytes were cultured for 9 days at 37°C in the presence or absence of metformin. At the end of this incubation period, the cells were incubated with minimally modified LDL (100 µg/ml) for 24 hours. The cells were then stained with 0.5% Oil Red O for 3 hours. Panel A shows representative phase-contrast photomicrographs from 3 independent experiments (magnification x 100). 1: control cells; 2: cells cultured with metformin 0.5 µg/ml; 3: cells cultured with metformin 1 µg/ml. Panel B shows cellular lipid accumulation as assessed by extraction of Oil Red O from stained cells and determination of the optical density. Data represent the mean ± SEM of 3 independent experiments.
Implications for clinical use of metformin

Animal studies reviewed above suggest that metformin may protect humans from the development of atherosclerosis. Supporting this possibility, results from clinical studies have demonstrated that metformin therapy is associated with reduced CV morbidity and mortality in patients with diabetes [8, 53]. The mechanisms of this protection are still poorly understood but may involve reduction of CV risk factors such as dyslipidemia, hypertension, and hypercoagulability as well as inhibition of AGE formation and oxidative stress [54, 55].

Conclusion

Experimental studies summarised in this review suggest that metformin exhibits antiatherogenic properties. This drug appears to prevent atherosclerosis through several mechanisms, including a reduction in lipid accumulation in the arterial wall. Additional mechanisms for the atheroprotective effect of metformin may include inhibition of major cellular events involved in atherogenesis, including leukocyte-endothelial interaction, foam cell formation, smooth muscle cell proliferation and platelet aggregation. One limitation of these studies is the use of wild type animals fed cholesterol-enriched diets. Future studies aimed at evaluating the effect of this drug in experimental models of diabetes and transgenic/knockout animal models are required to confirm these findings and demonstrate the potential for metformin therapy to attenuate the development of atherosclerosis in type 2 diabetes.

References