Antiplatelet Therapy, Diabetic Neuropathy and Peripheral Vascular Disease: A Unitary Approach

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Abstract

Although pathologically not necessarily the same, germane to both diabetic neuropathy and peripheral vascular disease is the morbid vascular complication of diabetes mellitus. As it appears, strict glycemic control alone is unable to neither prevent nor promote recovery of diabetic microvascular complications, thus the need to seek other treatment strategies. In addressing the vascular complications, a unitary approach between diabetic neuropathy, peripheral vascular disease and antiplatelet therapy is in order. Notably, the fine balance between platelet/coagulation and fibrinolysis systems are dominantly shifted to acceleration of the platelet/coagulation system in diabetes mellitus. Not only platelet homophilic aggregation, but also platelet heterophilic aggregation with neutrophils or monocytes in inflammatory conditions is observed. Hyperglycemia also induces overproduction of reactive oxygen species by multiple pathomechanisms that injure endothelial cells and reduce nitric oxide which eventually reduce the blood flow in microvessels. Among other antiplatelet agents, cilostazol is a novel antiplatelet agent that has been found to have beneficial effects in macrovascular events like stroke and peripheral vascular diseases. In peripheral vascular disease clinical trials, there is robust data that cilostazol is efficacious in improving walking speed. Also, cilostazol increases nerve blood flow/nerve conduction, and inhibits reduction in pericyte area of endoneurial microvessels in animal models of diabetic neuropathy. Clinical trials on diabetic polyneuropathy alone or in combination with peripheral vascular disease indicate clinical improvement in blood flow, but not necessarily in neuropathy parameters. Since a neurodegenerative process is equally as important a pathomechanism, the effect of antiplatelet therapy in diabetic neuropathy should be examined in long-term clinical trials that may potentially unfold its benefits over time.

Introduction

Microvascular complications characterized by retinopathy, nephropathy and neuropathy are well recognized in both type1 and type2 diabetes. Diabetic microvascular complications account for a significant portion of morbidity and mortality. In the past decade, intensive glycemic control and blood pressure control have shown to significantly reduce the microvascular related morbidity, however, this matter of prevention has not been borne out from large, long term clinical trials [1,2].

Whether stricter glycemic control alone is sufficient to prevent vascular complications in type2 diabetes is a matter of debate. In one clinical study [3], the intensive glucose lowering arm of the Action to Control and Cardiovascular Risk (ACCORD) trial in type 2 diabetes had been terminated because of increased mortality. In the said study [3] the targeted A1C level was below 6% and was combined with treatments to increase HDL cholesterol, lower LDL cholesterol and lower blood pressure. Another intensive glycemic control trial (Veteran Affairs Cooperative Study on Glycemic Control and Complications in Type II Diabetes Mellitus; VA-CSDM) [4] showed a non-significant increase in the risk of cardiovascular events. Furthermore, a long-term clinical trial for 5.6 years with intensive but relatively milder (targeted A1C 6.9%) glycemic control in type 2 diabetes (VADT) showed no significant effect on the rates of major cardiovascular events, death or microvascular complications, with the exception of progression of albuminuria [5]. These results suggest that strict glycemic control alone could not prevent or nor recover patients from diabetic microvascular complications, but may increase mortality. Stirban [6] suggested that: 1) lowering blood glucose in elderly and pluri-morbid patients should be performed with caution, 2) combination therapies should be rigorously considered, and 3) there may be a certain threshold beyond which lowering of blood glucose may be detrimental.

Along these conclusions, additive treatments with appropriate glycemic control will be needed for microvascular complications other than blood pressure control. In a multifactorial intervention study in type 2 diabetes for 13.3 years, intensive intervention with multiple drug regimens (antihypertensive drugs, statin, fibrate and aspirin) and behavior modification had sustained beneficial effects with respect to vascular complications, including microvasculopathy and on rates of death from any cause and from cardiovascular causes [7].

This present review highlights a unitary approach between the vascular complications such as diabetic neuropathy and peripheral arterial vascular disease (PAD), with the possible positive effects of antiplatelet therapy.

Platelet and Coagulation/Fibrinolysis Systems in Diabetes

Platelets participate in the blood haemostasis in case of blood

vessel injury accompanied by coagulation factors. The fibrinolysis system regulates the thrombus formation on endothelial cells resulting in maintenance of vascular homeostasis. The important concept of applying the platelet/coagulation system as well as the fibrinolysis system to vascular biology is that these fine systems always work even in healthy conditions, where endothelial cells are always damaged by shear stress under the blood flow and are always needed to be repaired by the same systems. Interestingly, this fine balance between the platelet/coagulation and the fibrinolysis systems are dominantly shifted towards the platelet/coagulation system acceleration induced by, among others, aging, hypertension, obesity, and especially by diabetic states. In addition to haemostatic factors, platelets contain several kinds of growth factors, such as vascular endothelial growth factor-A (VEGF-A), platelet derived growth factor (PDGF) and transforming growth factor-beta (TGF- beta). All of these growth factors have roles in vascular repair and maintenance. Platelet VEGF-A is a major component of serum VEGF-A [8] and serum VEGF-A significantly increases in patients with diabetic neuropathy during the neurologically active symptomatic stages [9]. These observations suggest the role of platelet VEGF-A on neuron homeostasis in diabetic neuropathy [9]. Pattern recognition receptors (RAGE [Receptor for Advanced Glycation Endproducts]) are known to be key molecules in a recent concept called "glucose memory" [10]. Hyperglycemia per se induces increased generation of advanced glycation end products (AGEs). The ligands for RAGE include AGEs, HMGB1 (high mobility group protein B1), and amyloid- beta -protein. RAGE is expressed on endothelial cells and its intra-cellular signaling leads to NF-kappaB activation, indicative of pro-inflammatory endothelial cell activation. From the recently reported RAGE expression on platelet membranes and since platelets are activated by AGEs through RAGE [10], a pathological link has been established between hyperglycemia, AGE, "glucose memory" and platelet activation. In effect, all these cascade of events may lead to a disturbance in microvascular circulation of patients with diabetes (Figure 1).

Platelet heterophilic aggregation in diabetes

From another aspect of platelet haemostatic function, a new concept of platelet biology has recently been developed. Not only platelet homophilic aggregation, but also platelet heterophilic aggregation with neutrophils or monocytes in inflammatory conditions are observed, and these heterophilic aggregations are closely associated with the innate immune system [11]. Ligand-receptor binding between Gram-negative bacterial Lipopolysaccharide (LPS) and Toll like receptor 4 expressed on platelet membrane activate platelets and form platelet-neutrophil aggregation [11]. On the other hand, platelet-monocyte aggregation by P-selectin/ PSGL-1 (P-Selectin Glycoprotein Ligand 1) binding leads to the stabilization of COX-2 mRNA in monocytes and prolongs the inflammatory milieu [12]. Hyperglycemia reportedly also induce both platelet-neutrophil and platelet-monocyte aggregation [13], and that AGEs reportedly induce platelet-neutrophil aggregation [14]. Although further studies may be required on the subject of platelet heterophilic aggregations in diabetic conditions, this new platelet biology of heterophilic binding to another type of cells suggest a close contribution to diabetic vascular complications, especially in understanding microvasculopathy.

De novo synthesis of platelet tissue factor in diabetes

Since platelets do not have a nucleus, platelets have been thought to have no capacity of protein de novo synthesis. Contrary to this previous concept, tissue factor (TF), which is an initiation factor of the extrinsic coagulation cascade, is produced in activated platelets through TF pre-mRNA splicing [15]. Gerrits and colleagues [16] reported that TF synthesis in platelets is inhibited by insulin in healthy individuals, but in patients with type 2 diabetes, inhibition is impaired. An increased amount of TF expression on platelets in patients with type 2 diabetes than in healthy individuals should be one of the mechanisms for macro- and microvascular complications of diabetes.

Platelet micro-particles in diabetes

Micro-particles derived from several cell lines including platelets and endothelial cells provide a new hypothesis for the pro-coagulant state in diabetic patients. Micro-particles are membrane fragments shed from damaged or activated cells. Platelet micro-particles (PMPs) bearing rich amount of TF in diabetic patients are strongly suggested to

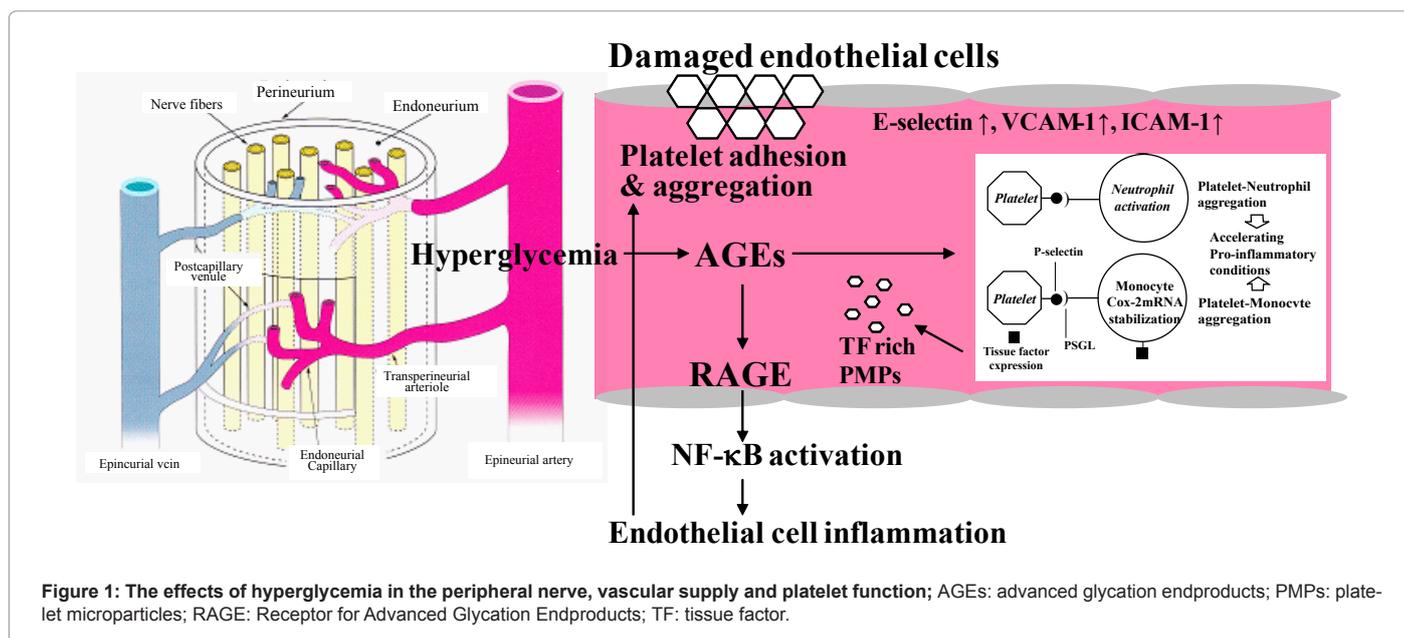

Figure 1: The effects of hyperglycemia in the peripheral nerve, vascular supply and platelet function; AGEs: advanced glycation endproducts; PMPs: platelet microparticles; RAGE: Receptor for Advanced Glycation Endproducts; TF: tissue factor.

promote the progression of macro- and microangiopathy [17].

Diabetic Neuropathy, Pathophysiology, and Antiplatelet Therapy

Risk factors and glycemic control in diabetic neuropathy

Vascular complications in diabetic neuropathy range from focal neuropathies (e.g. cranial nerves, femoral nerves) to symmetric polyneuropathies (DPN or diabetic polyneuropathy). All of the microvascular complications occurring in neuropathies (including autonomic neuropathy) in diabetic patients are categorically strong risk factors in the multivariable models, exceeding the effect of traditional risk factors [18]. In fact, previous studies indicated that peripheral neuropathy was related to mortality in diabetes [19,20]. These findings implicate that treatment of diabetic neuropathy as early as possible is highly recommended to prevent early mortality. The main strategy of the preventing neuropathy is believed to introduce a strict glycemic control. The strict glycemic control can decrease the incidence of macrovasculopathy and microvasculopathy, including retinopathy and nephropathy, but not necessarily neuropathy. The implication of these findings is that additive treatments other than glycemic control may be needed. Furthermore, intensive intervention with multiple drug regimens also could not entirely prevent nor allow recovery from neuropathy [7]. Thus, it is imperative that new treatment strategies ought to be developed in diabetic neuropathy.

Pathophysiology of diabetic neuropathy

The prevalence of diabetic neuropathy may approach approximately 50% in patients with longer-duration of illness. Chronic, symmetric sensori-motor polyneuropathy (DPN) and autonomic neuropathy are the most common forms of diabetic neuropathy. In the pathophysiology of DPN, direct metabolic dysfunction of cell and axon, as well as microvasculopathy, have been explored. Axonopathy are present in both myelinated and unmyelinated fibers, with early development of Schwann cell-axon networks, and later distal axonopathy. In tandem, alternations in microvessels are observed in peripheral nerves. Reduced blood flow due to autonomic nerve dysfunction may also contribute to the on-going neuropathic process [21]. It is obvious then that the pathophysiology of DPN is multi-factorial. The metabolic effects of chronic hyperglycemia and the consequences of ischemia on the peripheral nerve are likely to be critical factors that lead to neuro-axonal dysfunction and damage. Also, a wide range of pathomechanisms in DPN have been put forward that include activation of polyol pathway and protein kinase C (PKC), generation of reactive oxygen species (ROS) (oxidative stress), reactive nitrogen species, and AGEs [22]. Microvasculopathy in peripheral nerves are also multifactorial. Oxidative stress has been considered the final common pathway of cellular injuries in hyperglycemia. Several mechanisms of hyperglycemia-induced cellular injury were described in the vascular endothelium [23]. Hyperglycemia induces overproduction of ROS by the mitochondrial electron transport chain. Superoxides bind to nitric oxide (NO) that produce the strong oxidant peroxynitrite and injure endothelial cells. The reduced availability of endothelial NO causes the prevention of vasodilation which antagonizes thrombosis and anti-inflammatory properties. NO also inhibits the production of the potent vasodilator peptide endothelin-1.

Hyperglycemia is known to stimulate formation of diacylglycerol, which leads to the activation of PKC [24]. The oxidizing environment and increased carbohydrate accumulation in diabetes accumulate AGEs with the reduction of clearance from plasma [21]. The basement membrane of endothelial cells becomes glycosylated thereby contributing to impaired vasodilatation. Furthermore, AGEs bind to cell surface receptor for AGE (RAGE) and initiate an inflammatory signaling pathway that further increases oxidative stress and injures both neuronal and endothelial cells. These findings results in an imbalance of vasoactive agents and impair microvascular perfusion in nerves. A prospective study indicates that, apart from glycemic control, the incidence of neuropathy is associated with potentially modifiable cardiovascular risk factors, including a raised triglyceride level, body-mass index, smoking, and hypertension [25]. Linked to the vascular pathophysiologic mechanism of DPN, many treatment options have been proposed including a PKC-beta inhibitor, reduction of AGEs, VEGF and angiotensin converting enzyme inhibitors [22].

Antiplatelet therapy with cilostazol in diabetic neuropathy

A parallel involvement of both nerves and blood vessels may be the rule than the exception in diabetic neuropathy [26,27]. In the light of the foregoing issues of platelet involvement, antiplatelet therapy would figure as a likely candidate to improve the microvascular milieu in DPN. The 2-oxo-quinolone derivative cilostazol (Pletaal®) is a selective and reversible inhibitor of phophodiesterase 3A, with both antiplatelet and vasodilatory properties [28]. Cilostazol is a novel anti-platelet agent that has been found to have beneficial effects in macrovascular events like stroke and PAD.

Hinged on the vascular physiologic mechanism of DPN, a number of experimentally induced diabetes studies in animals demonstrated that cilostazol might have beneficial effects in that the drug inhibits platelet aggregation and leads to vasodilatation [28]. Through an improved nerve Na-K ATPase activity and cyclic adenosine monophosphate (cAMP) content, cilostazol increases nerve blood flow/nerve conduction, and inhibits reduction in pericyte area of endoneurial microvessels in animal models [29-35]. For instance, in a dose- and time-dependant manner, separate administrations of both locally applied and dietary cilostazol increased nerve blood flow [29], improved nerve conductions by restoring nerve Na-K ATPase activity [30,32,33], and allowed axonal regeneration [35]. While there are several published clinical trials (with reviews) on the benefits of cilostazol for PAD, such is not the case for the same drug in DPN [36]. Few published cilostazol studies in human participants with DPN are largely constrained by lack of randomization and comparator/placebo arms, selection bias, limited study population and lack of robust outcome parameters [33,37-39]. In addressing these main study issues, cilostazol was systematically studied in the most recent randomized placebo-controlled trial (ASCEND: Asian Study on Cilostazol Safety and Clinical Efficacy in Neuropathies of Diabetes Mellitus Type 2) [40], in which the primary efficacy parameter was a change in neuropathy symptom scores and the secondary efficacy parameter was a change in walking speed from baseline to week 12. The safety parameters were changes in nerve conduction studies as well as reporting of adverse events. The results indicate that despite a significant improvement in the neuropathy symptom scores in the overall motor and sensory categories of the 3 arms of the study (placebo, cilostazol low dose 100mg/day and high dose 200mg/day) from baseline to week 12, no significant differences were found among the groups. In this pilot Asian study, there is thus non-superiority of cilostazol in regard to improvement of neuropathy symptoms over placebo in the short study span. Interestingly, cilostazol, at low dose, was effective in improving walking speed from baseline to week 12, implying an improved blood flow. No significant worsening nor improvement in motor and sensory nerve conduction parameters were observed, Comparing the 3 study arms from baseline to weeks 4, 12, and 16, no significant worsening nor

improvement in motor and sensory nerve conduction parameters were observed, supporting cilostazol's safety. From the ASCEND study, one may be able to derive the following insights: (1) Cilostazol, with tight glycemic control, may have the added benefit of improving blood flow; (2) It may take time and a longer observation study period to possibly see effects of cilostazol in DPN, considering the trends (but not to significant levels compared to placebo) of improvement in motor and sensory neuropathy scores, and (3) Autonomic manifestations in DPN may have an independent pathophysiology, considering that it did not show improvement in the entire study period.

Another prospective double-blinded, randomized, placebo-controlled clinical trial assessed the effects of cilostazol on neuropathic symptomatology in patients with coexisting and established PAD and DPN [36]. In this perhaps the longest prospective study to date, no significant difference was found between the placebo and cilostazol groups, using the Toronto Clinical Neuropathy Scoring System and Vibration Perception Thresholds at 6 and 24 weeks. The common denominator between the ASCEND trial [40] and the aforementioned PAD and DPN trial [36] is the tight glycemic control and medically optimized cohort of patients enrolled in both studies.

Peripheral Artery Disease and Antiplatelet Therapy

Diabetic foot

Diabetic foot is an intractable complication of diabetes mellitus due to decrease in blood flow of lower limbs. Occurrence of diabetic foot is multifactorial, that includes presence of autonomic neuropathy, arteriosclerosis and concomitant infection due to reduced resistance to bacteria with hyperglycemia. If there is diabetes, the relative risk of PAD is 2-4 times higher than healthy subjects [25]. In diabetes, other independent risk factors for PAD including age, increased systolic blood pressure, reduced HDL cholesterol, smoking, prior cardiovascular disease, peripheral sensory neuropathy, and retinopathy, were also reported. Each 1% increase in A1c was associated with a 28% increased risk of PAD [41]. However, normoglycemic neuropathy subjects have significantly more features of metabolic syndrome (other than hyperglycemia) than diabetics [42]. Thus, it appears that neuropathy, one of the microvasculopathy in diabetes, is an important factor for PAD.

Endothelial dysfunction and PAD

Endothelial dysfunction due to hyperglycemia or insulin resistance may lead to the development of neuropathy [43]. Hyperglycemia induces a pro-coagulant effect irrespective of insulin levels, whereas hyperinsulinemia inhibits fibrinolysis irrespective of glucose levels [44]. These findings suggest that patients with hyperglycemia due to insulin resistance are especially susceptible to thrombotic events by a concurrent insulin-driven impairment of fibrinolysis and a glucose-driven activation of coagulation. In diabetes, the overlapping features of impaired vascular endothelium, platelet activation, increased activity of coagulation factors, changes in the structure of fibrin and decreased fibrinolytic activity, lead to a vicious cycle that further impair platelet function [45]. Thus, in the case of diabetic foot, glycemic control and vasodilator drugs (e.g. antiplatelet drugs, prostaglandin drugs) that improve blood flow, may be the key therapeutic approaches. While drugs which improve blood flow in diabetic neuropathy may be in the forefront, other approaches such as, gene therapy and cell therapy that promote angiogenesis may be another way in the future [46,47].

Antiplatelet drugs for PAD

According to both the Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II) and the American Cardiology and Heart Associations (ACC/AHA) guidelines, antiplatelet drugs should be administered for all patients with symptomatic PAD in order to reduce the risk of cardiovascular morbidity and mortality, (Grade A) [48]. Antiplatelet drug therapy for patients with PAD can be expected to be also effective prophylaxis of cardiovascular disease. Thus antiplatelet drugs will be used to manage all patients with PAD. Overall, among high risk patients, an allocation to antiplatelet therapy potentially reduce the combined outcomes of any serious vascular events and vascular mortality.

Aspirin: Low dose aspirin (75-150 mg daily) is an effective antiplatelet regimen for long term use in occlusive vascular events, but in acute settings, an initial loading dose of at least 150 mg aspirin may be required [49]. On the other hand, among 3350 participants without clinical cardiovascular disease, identified with a low ankle-brachial index (based on screening a general population-The Aspirin for Asymptomatic Atherosclerosis trial), the administration of aspirin compared with placebo did not result in a significant reduction in vascular events [50]. However, All asymptomatic patients and symptomatic patients with evidences of atherosclerosis in other circulatory beds should be prescribed an antiplatelet drug (Grade A) [25]. Asymptomatic patients without evidence for atherosclerotic disease elsewhere may be considered for antiplatelet therapy (Grade C) [25]. The ACC/ AHA guidelines recommend the administration of aspirin on individuals with or without clinical signs of other cardiovascular disease (Grade A) [51].

Clopidogrel: Clopidogrel is an inhibitor of platelet P2Y12 receptor similar to ticlopidine, but is considered a safer drug. In a randomized, blinded, trial of clopidogrel versus aspirin in patients at risk of ischemic events (CAPRIE), long-term administration of clopidogrel to patients with atherosclerotic vascular disease is more effective than aspirin in reducing the combined risk of ischemic stroke, myocardial infarction, or vascular death, and the overall safety profile of clopidogrel is at least as good as that of medium-dose aspirin [52]. Nevertheless, aspirin is generally considered the antiplatelet drug of choice because of the high incidence of coronary disease and its lower cost. As antiplatelet therapy like cilostazol, both aspirin and clopidogrel conceivably may be options for PAD, and perhaps even DPN. Apart from the fact that both lack vasodilatory effects, and if one were to pursue platelet aggregation methodological studies, occurrence of aspirin and clopidogrel resistance may reduce their respective efficacies [53].

Novel inhibitor of platelet P2Y12 receptor, prasugrel and ticagrelor are reported to have favorable effects after percutaneous coronary intervention compared with clopidgrel [54]. However, we await to this time whether these drugs will also achieve the desired effect in PAD, even if both drugs may have shown a stronger protection, efficacy and safety among diabetic patients.

Cilostazol: Cilostazol, a phosphodiesterase inhibitor, suppresses platelet aggregation and also acts as a direct arterial vasodilator. Cilostazol improved walking distances, significantly increasing initial claudication distance and absolute claudication distance [55,56]. Compared with placebo, long-term use of cilostazol, 100 mg or 50 mg twice a day, significantly improves walking distances in patients with intermittent claudication [57]. Based upon the evidence of benefit, a therapeutic trial of cilostazol is recommended to improve symptoms and increase walking distance in patients with claudication, particularly if previous antiplatelet agents were ineffective [25,51,58].

Conclusion

There leaves no doubt that platelet function and regulation do have important roles in diabetes mellitus per se, DPN and PAD. The jury has arrived as regard efficacy of cilostazol, more than other antiplatelet agents in the realm of PAD. However, in the realm of DPN, much work has yet to be established to reach robustness of efficacy. The situation changes with patients having coexistent PAD and DPN, both at different stages together. An improved blood flow with antiplatelet therapy may either be delayed or not be reflected in nerve blood flow and thus not transmute to alleviation of neuropathy symptoms. Moreover, one should be aware that in DPN in particular, not only vascular but neurodegenerative mechanisms (whether in association with metabolic disturbances or not) come into play, such that there may be an inconsistent or perhaps delayed response to medications.

Acknowledgement

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