Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

Brain Research

Research Report

Microparticles generated during chronic cerebral ischemia increase the permeability of microvascular endothelial barriers in vitro

Hamidreza Edrissi[a], *Sarah C. Schock*[b], *Antoine M. Hakim*[b], *Charlie S. Thompson*[b,*]

[a]University of Ottawa, Neuroscience Graduate Program, 451 Smyth Road, Ottawa, ON, Canada K1H 8M5
[b]Ottawa Hospital Research Institute, Neuroscience, 451 Smyth Road, Ottawa, ON, Canada K1H 8M5

ARTICLE INFO

Article history:
Accepted 16 December 2015
Available online 23 December 2015

Keywords:
Cerebral ischemia
Endothelial barrier permeability
Microparticles
TNF-α
Rho kinase
Apoptosis

ABSTRACT

Numbers of circulating microparticles (MPs) are elevated in a variety of cardiovascular disorders, and recent studies indicate that they are involved in inflammatory intercellular signaling. In the present study the signaling properties of MPs were assessed in an in vitro model of the blood brain barrier. MPs isolated from the plasma of rats exposed to chronic cerebral ischemia caused a significant reduction in the transendothelial electrical resistance (TEER) when applied to in vitro endothelial barriers, while MPs isolated from an equal volume of plasma from unoperated or sham operated rats did not. The reduction in TEER was attenuated by treating endothelial barriers prior to exposure to MPs with the caspase 3 inhibitor AC-DEVD-CHO, the TNF-α inhibitor SPD304, the tumor necrosis factor alpha-converting enzyme (TACE, ADAM 17) inhibitor TAPI-0-1 and the Rho kinase (ROCK) inhibitor Y-27632, and by treating the MPs themselves with these inhibitors prior to applying them to cultured cells. This observation indicates that MPs generated during cerebral ischemia contain pro-TNF-α, active TACE and active ROCK. ROCK and Ras homolog gene family member A (RhoA) were detected in MPs by western blot. The growth factor VEGF stimulated transcellular transport in endothelial barriers while exposure to MPs did not. We conclude that the increase in permeability of artificial barriers induced by MPs is primarily due to enhanced apoptosis induced by activation of the TNF-α pathway and activated caspase 3 and Rho kinases delivered to endothelial cells by MPs.

1. Introduction

Cognitive impairment is known to accompany a variety of neurodegenerative disorders and there is substantial evidence to suggest that dysfunction of the blood brain barrier is an early pathological event underlying this condition (Hermann et al., 2014). Microparticles are small, membrane bound extracellular vesicles, 0.1 to 1.0 μm in size that are released into the extracellular space from different cell types under normal and pathologic conditions (György et al., 2011;

*Corresponding author.
E-mail address: charliet@uottawa.ca (C.S. Thompson).

http://dx.doi.org/10.1016/j.brainres.2015.12.032
0006-8993/

Italiano et al., 2010) and are now recognized as important mediators of intercellular signaling. MPs contain membrane and cytoplasmic molecules of their cell of origin and their composition may depend on stimuli that lead to their generation. MPs transfer information from the cell of origin to target cells by MP-to-cell contact or through secretion of soluble mediators (Mause and Weber, 2010).

MPs in the plasma originate from multiple cell types and their relative concentrations and properties are determined by the ambient physiological condition. Circulating MPs have proven to be biomarkers of vascular injury and inflammation in cardiovascular pathologies such as acute myocardial infarction, hypertension, and atherothrombosis (Lovren and Verma, 2013), as well as a variety of neurological disorders including ischemic cerebrovascular accidents, transient ischemic attacks, multiple sclerosis, cerebral malaria (Doeuvre et al., 2009; Marcos-Ramiro et al., 2014a, 2014b), and Alzheimer's disease (Xue et al., 2012).

The endothelium is one of the main targets of circulating MPs and MPs contribute to the regulation of endothelial cell function under physiological conditions (Owens and Mackman, 2011). However, MPs released from cells under pathological conditions may have different properties and induce a pro-coagulant, pro-inflammatory phenotype that leads to endothelial dysfunction (Diamant et al., 2004). Endothelial dysfunction is a key element in progression of atherosclerosis and subsequent stroke occurrence (Jung et al., 2009), and has an important role in the etiology of symptomatic cerebral small vessel disease (CSVD, Lavallée et al., 2013). Pantoni and Garcia (Pantoni and Garcia, 1997) have proposed that an initial step in the pathogenesis of CSVD might involve a failure of the arteriolar endothelium due to partial ischemia. Endothelial dysfunction could cause the pathological symptoms of CSVD in two ways. The reduction in baseline cerebral blood flow and impaired autoregulation and reactive hyperemia may predispose small regions of the brain parenchyma served by single vessels to become ischemic (O'Sullivan et al., 2002; Farkas et al., 2006). Alternatively, an increase in blood–brain barrier (BBB) permeability may result in leakage of plasma components into the vessel wall and brain parenchyma, causing inflammation, thickening of the vessel wall, lesions in the parenchyma and a further reduction in blood flow (Wardlaw, 2005; Wardlaw et al., 2009, 2008, 2003).

In the present study the properties of circulating MPs generated during cerebral ischemia were studied using an in vitro model of the BBB formed by cultured rat brain microvascular endothelial cells (RBMVECs). MPs were isolated from the plasma of rats exposed to chronic cerebral ischemia by permanent bilateral common carotid occlusion (BCCAO). This model produces a chronic reduction in cerebral blood flow by 50% to 70% resulting in white matter lesions with vacuolation of myelin, axonal damage, and demyelination in corpus callosum, internal capsule, and caudate putamen (Ohta et al., 1997; Ueno et al., 2009; Wakita et al., 1995; Wakita et al., 2002; Farkas et al., 2004). We found that exposure to circulating MPs causes a large increase in barrier permeability that is not due to stimulation of the caveolae-mediated transcellular pathway. MPs cause an increase in levels of activated caspase 3 and activation of the TNF-α and Rho/ROCK signaling pathways in RBMVECs and MPs themselves contain pro-TNF-α, active TACE, RhoA and ROCK. The increase in permeability in in vitro RBMVEC barriers appears to be due primarily to apoptosis.

2. Results

Following treatment of confluent RBMVECs with MPs isolated from 2VO, sham or unoperated rats for 24 h, only MPs from ischemic rats caused a significant decrease in TEER compared to control. The decrease was also significantly greater than that caused by MPs from unoperated or sham operated rats (Fig. 1A, treatment effect: F (3, 7)=4.95 ($P<0.05$), time effect: F (10, 70)=29.78 ($P<0.0001$), time*treatment effect: F (30, 70)=5.54 ($P<0.0001$)). MP treatment of confluent RBMVECs initially induces increased F-actin fiber formation and disassembly of these fibers and adherens junctions at 8 and 24 h (Fig. 1B). Following exposure to MPs there was often a transient increase in TEER, that correlated in time with increased formation of actin stress fibers and subcortical bundles (Fig. 1C, treatment effect: F (1, 3)=24.85 ($P<0.05$), time effect: F (1, 3)=23.95 ($P<0.05$), time*treatment effect: F (3, 3)=69.01 ($P<0.01$)).

In order to assess the role of apoptosis in the decrease in TEER, confluent RBMVECs were treated with the caspase-3 inhibitor AC-DEVD-CHO prior to exposure to MPs (Fig. 2A) and, in a separate experiment, MPs were treated prior to being added to the cultures (Fig. 2B). In both cases there was a significant reduction in the increase in barrier permeability (2A, treatment effect: F (3, 7)=61.735 ($P<0.001$), time effect: F (10, 70)=67.24 ($P<0.0001$), time*treatment effect: F (30, 70)=17.83 ($P<0.0001$) and 2B, treatment effect: F (3, 7) = 8.89 ($P<0.01$), time effect: F (10, 70)=11.25 ($P<0.0001$), time*treatment effect: F (30, 70)=3.48 ($P<0.0001$). Pretreatment of confluent RBMVECs with AC-DEVD-CHO before MP treatment reduced disruption of adherens junctions (Fig. 2C). TUNEL staining confirmed that DNA fragmentation occurs following exposure to MPs (Fig. 2D). Note that F-actin staining is in the nucleus following exposure to MPs.

MPs generated during cerebral ischemia have been shown to act as vectors of TNF-α signaling (Schock et al., 2014) and TNF-α is known to affect brain endothelial cell barrier permeability (Wiggins-Dohlvik et al., 2014). SPD-304 prevents trimerization of soluble TNF-α and binding to a TNF-α receptor (He et al., 2005). Addition of SPD-304 to culture medium prior to addition of MPs causes a significant reduction in the enhanced permeability in artificial barriers, (Fig. 3A, treatment effect: F (3, 7)=9.55 ($P<0.01$), time effect: F (10, 70)=74.37 ($P<0.0001$), time*treatment effect: F (30, 70)=4.45 ($P<0.0001$)) and, in cultured cells, less disruption of adherens junctions (Fig. 3B). TNF-α converting enzyme (TACE/ADAM17) is a sheddase located in the plasma membrane of cells and one function of TACE is to cleave transmembrane pro-TNF-α to release a soluble TNF-α ligand (McCoy and Tansey, 2008). When MPs are treated with TAPI-0 (a small molecule inhibitor of TACE) prior to being added to artificial barriers or cultured RBMVECs there is a significant reduction in the permeability increase, (Fig. 3C ,treatment effect: F (3, 7)=0.51 ($P<0.05$), time effect: F (10, 70)=37.06 ($P<0.0001$), time*treatment effect: F (30, 70)=8.80 ($P<0.0001$)) and protection of adherens junctions and F-