Eur J Vasc Endovasc Surg (2018) ■, 1–10

Catheter Injected Bone Marrow Mesenchymal Stem Cells Induce Efficacious Occlusion of Arteriovenous Nidus in a Swine Model

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WHAT THIS PAPER ADDS

This study presents an original cell therapy approach addressing arteriovenous malformations (AVM) in an animal model previously used for acute evaluation of thrombotic agents (rete mirabile in swine). Bone marrow derived mesenchymal stem cells were delivered to the reticular vascular structure, after incorporating cells into a hydrogel allowing for their sustainable presence. Animal survival allowed chronic assessment of the AVM, showing microvascular occlusion of nidus through endoluminal solid, cellular and collagenic tissue growth. The possibility of injecting living cells into a microvascular network in a chronic survival model provides tools for research and treatment of AVMs and potentially other microvascular diseases.

Objectives: Arteriovenous malformations (AVMs) are complex vascular lesions. Surgical excision is the treatment of choice, but is often not achievable. Embolo-sclerotherapy alone is associated with high recurrence rates. This study tested the hypothesis that seeding hydrogel conditioned bone marrow derived mesenchymatous stem cells (BM-MSCs) in an AVM nidus model induces solid microvascular occlusion through endoluminal tissue growth.
Methods: AVMs were modelled as arteriovenous microvascular nidus, using swine rete mirabile, a plexiform intracranial structure composed of arterial microvessels that extensively anastomose. A right carotid-jugular fistula was created to generate high flow in the rete, and bone marrow was aspirated. At day 14, cultured BM-MSCs marked with a red fluorochrome were incorporated into a hyaluronic acid hydrogel, and injected through a catheter into the rete mirabile, using femoral access. In specific groups microsphere embolisation immediately preceded gel injection. At day 28, the swine were euthanased and the rete mirabile harvested for qualitative and quantitative analysis of microvessel lumen occlusion.
Results: Actual transfer of PKH26 labelled cells in rete was confirmed. In a first phase of the study, five swine died as a result of neurological events, prompting reductions of the injected volumes. Twenty-three animals survived until day 28. Injection of BM-MSC loaded hydrogel (n=6) significantly increased the occlusion rate compared with injection of acellular hydrogel (n=7) (10% [range, 10–12%] vs. 26% [range, 20–41%], p=.016). Injection of BM-MSC loaded hydrogel immediately after microspheres (n=6) enhanced the occlusion rate compared with embolic microspheres alone (n=6) (50% [range 46–56%] vs. 22% [range, 15–27%] p=.045). Microsphere injection resulted in vascular luminal thrombus formation. Injection of BM-MSCs induced endoluminal growth of vascular smooth muscle cells with cell proliferation and a dense collagen rich extracellular matrix.
Conclusion: The luminal occlusion pattern of a microvascular AVM like structure can be changed from thrombus to solid cellular accumulation. The possibility of injecting living cells in a microvascular network, in a chronic survival model, provides new tools for research and treatment of AVMs and other microvascular diseases.

Article history: Received 2 July 2017, Accepted 7 December 2017, Available online XXX
Keywords: Arteriovenous malformations, Mesenchymal stem cells, Cell therapy, Embolisation

INTRODUCTION

Arteriovenous malformations (AVMs) are complex congenital vascular lesions defined by the presence of one or multiple arteriovenous shunts. They result from a defective development of arterial and venous systems during embryogenesis.[1–3] Most AVMs belong to the extra-truncular group, in which embryogenetic dysfunction occurs at an early stage of vascular development, leading to

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1078-5884/
https://doi.org/10.1016/j.ejvs.2017.12.011

abnormal communications between multiple dysplastic arterial and venous vessels, instead of maturing into a normal capillary bed. The resulting plexiform high flow lesion is usually termed "nidus."[4] Unlike truncular AVMs originating from a later stage of embryogenesis, extra-truncular forms possess unique embryological characteristics related to the persistence of progenitor cells, resulting in dynamic behavior and rapid growth in case of hormonal or traumatic stimulation (e.g. hormone, menarche, pregnancy, surgery, trauma), and in recurrence in case of incomplete treatment.[5–7] This dynamic behavior is mediated by multiple chemokines and transcription factors with various expression patterns depending on the clinical stage of the AVM.

The current consensus on treatment is complete surgical resection of the AVM, usually preceded by flow limiting embolisation. However, these lesions are sometimes surgically inaccessible, their boundaries are poorly defined, and/or removal encompassing free margins is highly destructive.[4] Embolo-sclerotherapy alone in these situations is hampered by a high recurrence rate, and is considered to be a palliative rather than curative approach.[8,9] Recurrence after embolisation is thought to be mediated in part by nidus repermeabilisation when upstream thrombus formation after embolisation procedures is countered by local fibrinolysis. Reported high recurrence rates are a major limitation to embolisation, either pre-operatively or for surgically inaccessible lesions.

Facing the obvious lack of both curative and safe treatment for many patients, with high impact on quality of life, organ function, and sometimes survival, the present study tested the hypothesis that the lumen of the nidus could be occluded, not by thrombus subjected to fibrinolysis and repermeabilisation, but by solid material.

Bone marrow derived mesenchymal stem cells (BM-MSCs) are multipotent cells that can be easily isolated and cultured. They can differentiate into multiple cell types derived from mesodermal lineage, and have been used by the present study group to fill endovascular spaces by neo-vascular tissue formation after catheter guided injection.[10]

This study tests the hypothesis that catheter-directed injection of BM-MSCs conditioned in a hydrogel, into the nidus of an AVM model in pig, induces vascular lumen occlusion through endoluminal tissue growth.

MATERIAL AND METHODS

Animals were housed and taken care of according to the European directive for animal care (2010/63/EU).

Study design

The model of AVM as described by Massoud[11] was used, based on flow diversion across swine rete mirabile (Fig. 1). Rete mirabile is a median plexiform microvascular network in swine, located at the base of the skull, that shares morphological and histological similarities with an AVM nidus. The plexus of microarteries that make up the rete mirabile extensively anastomose with an individual rete vessel diameter ranging from 70 to 700 µm, when the average size of a human AVM nidus vessel is 265 µm.[11] It follows the end division of the right and left ascending pharyngeal arteries, collaterals of the common carotids. Unlike an AVM nidus, there is no direct communication inside the structure of the rete mirabile between

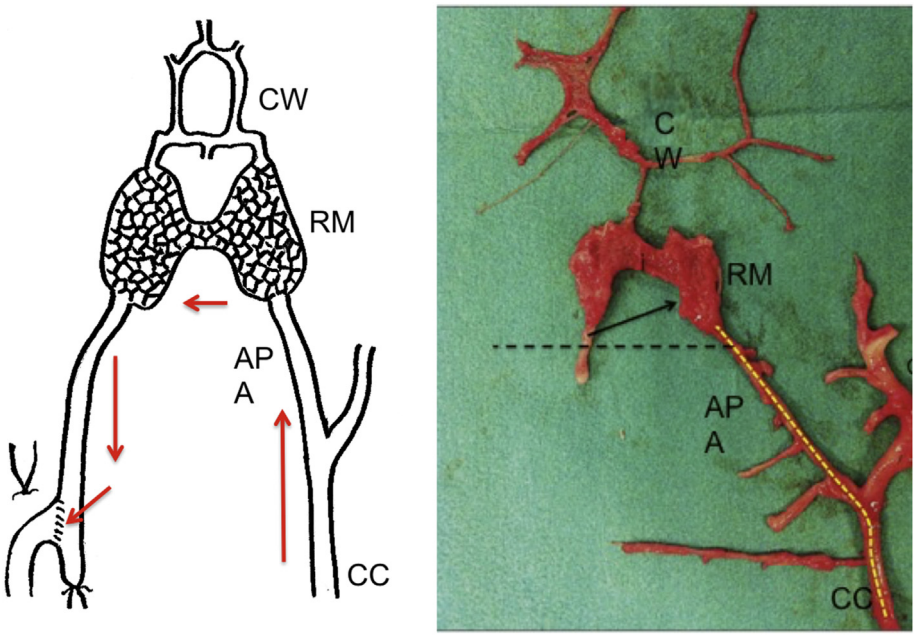

Figure 1. Diagram showing flow diversion model across the rete mirabile as described by Massoud et al.[11] using unilateral carotid-jugular fistula. En bloc removal of the rete mirabile with its feeding vessels. CC = common carotid; APA = ascending pharyngeal artery; RM = rete mirabile; CW = Circle of Willis. The yellow dotted line shows the catheter direction during phase 2. The black dotted line represents the level of the base of the skull.

Please cite this article in press as: Touma J, et al., Catheter Injected Bone Marrow Mesenchymal Stem Cells Induce Efficacious Occlusion of Arteriovenous Nidus in a Swine Model, European Journal of Vascular and Endovascular Surgery (2018), https://doi.org/10.1016/j.ejvs.2017.12.011