Endovascular Treatment with Platinum Coils

Recanalization is Associated with Early Increased von Willebrand Factor mRNA

J. RAYMOND, C. OGOUDIKPE, A. METCALFE, I. SALAZKIN, G. GEVRY, O. ROBLEDO

CHUM Research Centre – Notre-Dame Hospital, Montréal, Québec, Canada

Key words: aneurysms, endovascular treatment, radiation, endothelial denudation, growth factors, metalloproteinases, experimental models

Summary

Recanalization after coil occlusion is a concern for long-term results of endovascular treatment. Knowledge of molecular events following coil occlusion and recanalization could help design specific strategies to promote permanent occlusion.

Platinum coils were implanted into canine maxillary, vertebral or lingual arteries. Coil occlusion (treatment 1), routinely followed by recanalization was compared with two strategies to prevent recanalization: beta radiation using ^{32}P coils (treatment 2) and endothelial denudation, using an endovascular device, followed by coil occlusion (treatment 3).

The evolution of initial complete occlusions was followed by angiography and pathology at three months. Levels of messenger RNA of vWF (von Willebrand factor), SMA (smooth muscle actin), CD14, CD31 (or PECAM-1: Platelet Endothelial Cell Adhesion Molecule-1), PDGF-BB (platelet-derived growth factor), TGF- β 1 (transforming growth factor), MCP-1 (macrophage chemoattractant protein), Angiopoietins, Metalloproteinases-9, 14 and inhibitors (TIMP-2, 4) were followed by Reverse Transcription and Polymerase Chain Reaction (RT-PCR). Analyses were performed one, four, seven and 14 days after coiling, and levels of expression after the three treatments were compared using ANOVAs.

Intact arteries treated with platinum coils routinely recanalize (100%), but arteries treated by denudation and coiling or with radioactive coils recanalize in only 17% and 4% respectively ($P < .001$). Recanalization was associated with increased levels of vWF mRNA at seven days, a finding that was not observed with denudation or radiation ($P = .015$). There was no other significant difference.

Recanalization is associated with early vWF expression, perhaps reflecting the development of endothelialized channels through thrombus formed after coil occlusion.

Introduction

Potential benefits of coil embolization of ruptured intracranial aneurysms may be jeopardized by the occurrence of angiographic recurrences, a persisting concern for future hemorrhages^{1,2}.

Recurrences are more frequent when residual flow persists at the neck of incompletely treated aneurysms², but even when angiographic occlusion is documented in certain animal models, recanalization occurs routinely after coil embolization^{3,4}. Coil embolization leads to occlusion through the formation of thrombus, and this thrombus is organized into a neointima sealing the neck of aneurysms, or recanalized through a cellular process that can be inhibited by in situ beta radiation^{3,4}, or by

endothelial denudation prior to coil deposition^{5,6}.

There is little information regarding molecular expression associated with organization and healing, or recanalization and recurrence after coil occlusion. A better understanding of presumably more fundamental molecular phenomena might permit their modulation to improve the long-term outcome of endovascular treatment of aneurysms⁷.

The expression of numerous molecules could have been explored in the current study. In the absence of an accepted paradigm to orient research we reviewed various vascular pathophysiological models in a search for analogies. Organization and recanalization of thrombus may share conceptual mechanisms with neointima formation and angiogenesis respectively (reviewed in⁷). Thus we chose to address first mRNA levels of endothelial and neointimal cell markers, growth factors as well as metalloproteinases and their inhibitors reported to be involved in these phenomena.

A comparative exploration of gene expression in our arterial model in which constant recanalization after coil occlusion can reliably be prevented by two different approaches could identify key molecular events responsible for, or at least associated with, the favourable or unfavourable evolution of therapeutic occlusions.

Material and Methods

All experimental procedures were approved by our Institutional Animal Care and Use Committee and performed in compliance with guidelines of the Canadian Council on Animal Care. All procedures were performed under general anaesthesia. Beagles weighing 10 to 15 kg were sedated and anaesthetized as described³⁻⁶.

Arterial occlusion model

This model has previously been described^{3,4}. Briefly, a percutaneous femoral puncture was used to reach bilateral maxillary, lingual or vertebral arteries with 2F microcatheters. Single coil occlusion of these arteries leads to thrombosis by one hour followed by recanalization by two weeks^{3,5}. Angiographic and pathological findings are identical whether arteries are maxillary, lingual or vertebral^{3,6}. A 3 mm calibre 0.015 platinum coil 8 cm in length (Boston Sci-

entific) was implanted into a 10 mm segment of each artery (treatment 1). Recanalization was inhibited using two different strategies: in situ beta radiation using ³²P coils (treatment 2) or denudation of the endothelial lining (treatment 3)³⁻⁶. Treatments were allocated in a paired fashion (permutating right and left) for each strategy (radioactive vs. non-radioactive coil; platinum coil in denuded artery vs. coil in normal artery) as well as for controls (denudation only or no treatment) while locations (maxillary, lingual or vertebral) of paired comparisons were permuted from one animal to the other. In one group of animals ³²P coils (treatment 2) were compared to platinum coil embolization (treatment 1). ³²P coils were produced as previously described on a dedicated radioactive ion implanter^{3,4}. ³²P was purchased from Perkin Elmer Life Sciences. Coils were measured after implantation, identified and sterilized. Mean coil linear activities were $0.023 \pm 0.018 \mu\text{Ci/cm}$.

In another group of animals, platinum coil embolization (treatment 1) was compared to denudation and coil occlusion (treatment 3). Denudation of the arterial wall was accomplished using an aneurysmal neck-bridge device (ANBD)^{8,9}. This platinum covered nitinol device was designed to assist coiling of wide-necked aneurysms. It is composed of three loops 3 mm in diameter attached by a common stem. Typically five passes with rotations were performed in each arterial segment in an effort to scrape off the endothelial lining.

For molecular analyses, the main object of this publication, performed at one, four, seven and 14 days, we studied 24 arteries that were denuded and coiled, 56 arteries that were occluded with platinum (40) or radioactive (16) coils, without denudation, and 16 were denuded with the ANBD but not coiled. Sixteen intact arteries served as baseline controls.

Four to eight specimens per time point were used for RT-PCR analyses. To document the recanalization rates of arteries submitted to these different treatments, we studied a total of 124 arteries (73 occluded with radioactive coils, 39 with non-radioactive coils and 12 denuded and coiled) three months after embolization by angiography and macroscopic photography of pathology specimens. These animals were initially dedicated to the study of the safety of ³²P-coils compared to platinum coils, which will be the subject of another publication¹⁰. Angiogra-

Figure 1 Pathology of recanalization or its inhibition. Microscopic (A-E) or macroscopic findings (F-H) 7 (A,B) or 90 days (C,H) after occlusion with platinum (A,C,F) or radioactive coils (B,E,H) or coil occlusion preceded by denudation (D,G). Note: channels lined by endothelial cells (arrows in A,C), absent when coils are radioactive (B). HPS (A,B), Movat's pentachrome (C-E); original magnification x400 (A,B) or x100 (C-E).

phy was performed at the end of the procedure and immediately before sacrifice, if performed at seven days or more. Multiple projections following selective injections were interpreted in a blind fashion. Occlusion was defined as the absence of antegrade blood flow through the arterial segment. Any antegrade contrast opacification was sufficient to label the artery recanalized.

Macroscopic photography and pathology

Macroscopic and microscopic stereophotographs of cut sections of arteries were performed using a computerized imaging system (Clemex, Longueuil, Quebec). Organization and recanalization were evaluated at three months

according to a qualitative scoring system^{8,11}. Briefly, sections were labelled score of 0 when dense conjunctive tissues occluded the lumen; score one when organized tissues presented small areas of recanalization; score two when a large crescent of recanalization was present; score three when recanalization of the lumen was complete, with a thin neointima covering the coils; and score four when only unorganized thrombus was found. We also more simply evaluated the specimens regarding patency (patent vs occluded). Arteries were studied after formalin fixation, coil removal by micro dissection, axial sectioning, and staining with hematoxylin-phloxine-saffron (HPS) and Movat's pentachrome stain, as described³⁻⁵. Im-

Table 1 PCR primer sequences of selected genes.

	Gene	Tm	NT(bp)	Forward primer	Reverse primer	Type
Cellular Markers	vWF	64	1057	5'-CGGATCCGCGTGGCTGTGGTG-3'	5'-CTCCAGAGCAGACCTCTGTGA-3'	Dog (NM-001002932)
	CD31	52	687	5'-CCCAGGGTGACACTGGACAA-3'	5'-CCTTCTGGATGGTGAAGTTGG-3'	Consensus human/mice
	SMA+	52	510	5'-AAGGCCAACCGGGAGAAAATGAC-3'	5'-TTCCTGATGTCATATATCACACTTC-3'	Consensus human/mice
MMPs	CD14	64	455	5'-CCTCCGCAACGTGTCGTGGGC-3'	5'-AGCACGCTGAGCTTGGCTGGCAG-3'	Consensus human/mice
	MMP-2	58	580	5'-ATGCAGAAAGTTCTTTGGGCTGC-3'	5'-TTGCCATCCTTCTCATAAGTTGTA-3'	Dog (AF177217)
	MMP-9	64	476	5'-TATGACACCGACCGTCCGGTTC-3'	5'-GTACATGAGCGCTTCTGGCAC-3'	Dog (AF 169244)
	MMP-12	58	245	5'-TGCTTTTGATGGCAAAAGGTGG-3'	5'-TCATCAGCAGAGAGGGGAAATGTG-3'	Consensus human/mice
	MMP-14	64	420	5'-CCTACGAGAGGAAGGATGGCA-3'	5'-CTCATCTGGCCGGCTCCCCGA-3'	Dog (AY 534615)
	TIMP-1	58	345	5'-GCGTTATGAGATCAAGATGAC-3'	5'-CTGGTCCGTCCACAAAGCA-3'	Dog (NM-001003182)
TIMPs	TIMP-2	58	660	5'-ATGGGCGCGCGGCCCGCAGCCT-3'	5'-CTTATGGGTCTCTCGATGTCGAGAAA-3'	Dog (NM-001003082)
	TIMP-3	58	298	5'-TACCAGTACCTGCTGACAGG-3'	5'-GCCCATCTCGGTACCAGCT-3'	Consensus human/mice
	TIMP-4	58	331	5'-ATCTCCAGTGAGAAAGGTAGT-3'	5'-GTGGTGATTGGCAGGCCACAGTTTC-3'	Consensus human/mice
Growth Factors	VEGF	52	620-548-488	5'-TTCTGTATCAGTCTTCTCTGGTGAG-3'	5'-CGAAAGTGGTGAAGTTTCATGGATG-3'	Human (ref: Matsunaga T. Circ. 2000;102(25):3098-3103)
	Ang-1	52	150	5'-GAAGTCTAGATTTTCCAAAGAG-3'	5'-GGTTTCTCTCCAGTCCATT-3'	Dog (AF 345932)
	Ang-2	52	544	5'-GAGATCAAGGCCTACTGTGAC-3'	5'-GATCATCATGGTTGTGGCCTT-3'	Consensus human/mice
	PDGF-BB	64	393	5'-ATGAATCGCTGCTGGGCGCTCTTCCT-3'	5'-GGAGCAGCGCTGCACCTCCAC-3'	Dog (NM-001003383)
	TGF- α	64	386	5'-TTCCTGCTCCTCATGGCCAC-3'	5'-GCAGGAGCGCACGATCATGT-3'	Dog (NM-001003309)
	MCP-1	64	305	5'-ATGAAGGTCTCCGACGCGCTC-3'	5'-TCATGGCTTTCAGTTTGGGTTTG-3'	Consensus human/mice
Reporter	TNF- α	64	436	5'-CCTCCAACTAATCAGCCCTCT-3'	5'-TCTCAGCGCTGAGTCGATCA-3'	Dog(NM-001003204)
	GAPDH	64	400	5'-ATGGTGAAGGTCGGAGTCAAC-3'	5'-CATCACAAACATGGGGGCATCAGC-3'	Dog (NM-001003142)

vWF: von Willebrand factor; SMA: smooth muscle actin; MMP: Metalloproteinases; TIMP: Inhibitor of metalloproteinases; VEGF: vascular endothelial growth factor; Ang: angiopoietin; PDGF-BB: platelet-derived growth factors; TGF- β : transforming growth factor beta-1; MCP-1: macrophage chemoattractant protein; TNF- α : Tumor necrosis factor; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

munohistochemistry served to characterize cells using antibodies to smooth muscle α -actin and factor VIII^{3,5}.

RT-PCR analysis

Isolation of total cellular RNA was performed using the Micro-to-Midi system (Invitrogen) according to the manufacturer's instructions. Aliquots of total cellular RNA (500 ng) were used for first strand cDNA synthesis in 20 μ l reaction volume using Superscript II reverse transcriptase. After determining the linear range of PCRs for each of the target genes, amplification of the gene under investigation was carried out using the primers summarized in table 1 and chosen in two different exons to distinguish genomic contamination. PCR amplifications were performed with Platinum Taq DNA polymerase according to the manufacturer's instruction on an Eppendorf Mastercycler gradient using the following program: step 1, 94 °C for one min; step 2, between 52 and 64 °C for one min; and step 3, 72 °C for one min (table 1). Forty cycles were performed for the amplification of genes of interest and 30 cycles for GAPDH. The amplification for each gene was in the linear curve. PCR products were visualized on 1.5% agarose gel stained with ethidium bromide and UV trans-illumination. Quantitative analysis was carried out using a computerized densitometric imager (ImageQuant) to obtain genes/GAPDH ratios.

Statistics

Angiographic occlusion rates and macroscopic photographic results were compared using Kruskal-Wallis test. mRNA levels, analysed by densitometry, were compared with analysis of variance followed, if necessary, by Tukey's contrast, taking into consideration the effects of time and groups of treatment. To control the false discovery rate, the Benjamini and Hochberg correction was used. For all statistic tests, $P < .05$ was considered statistically significant.

Results

1. Angiographic results

All arteries treated by coils, radioactive or not, were occluded at the end of the procedures. Arteries treated by denudation only showed some minimal spasm but no occlusion. For the group of animals used for biochemical

analyses, normal arteries treated with non-radioactive coils recanalized by 14 days (6/6), but some as early as seven days after embolization (3/8). Arteries treated by denudation and coil deposition remained occluded at seven (8/8) or 14 days (6/6). Arteries treated with radioactive coils remained occluded at seven (8/8) and 14 days (6/6).

At three months recanalization rates were 100%, 17% and 4% ($P < .001$) for non-radioactive coiling, coiling and denudation and embolization with radioactive coils respectively, in the group of animals used to assess the evolution of occlusion three months after treatments.

2. Pathology and macroscopic photography

a) Microscopic analysis

Very early specimens (<7 days) were degraded by the friability of the clot and the artefacts caused by the extraction of the metallic coils. By seven days recanalized channels in arteries treated with platinum coils could be recognized (figure 1A). They were endothelialized and surrounded by myofibroblasts in a fibrous matrix. With time there was an increasing content of collagen. Arteries treated by denudation or radioactive coils did not show early endothelialized channels (figure 1B). At three months, arteries treated with radioactive coils or by denudation and coiling were occluded with conjunctive tissue of the same nature as the one found in recanalized arteries treated with platinum coils (figure 1C-E).

b) Photographic scores

At three months arteries treated with radioactive coils were more completely occluded and more organized than arteries treated with platinum coils or by denudation and coiling ($P < .005$; figure 1F-H). Arteries treated with denudation and coiling were not significantly different from arteries treated only by coiling in regard to organization (figure 1G).

3. Gene expression

a) Genes associated with platinum coil occlusion and evolution with time and recanalization

Coil occlusion was followed by increased expression of some endothelial (vWF, CD31) and monocyte-macrophage markers (CD14), returning to control levels by 14 days except for CD14 (figure 2A). Statistical differences were found between days for vWF that increased to be maximal on day seven ($P = .020$) and SMA

Figure 2 Genes associated with platinum coil occlusion and evolution with time. Results of RT-PCR analyses, expressed as specimen/control artery ratio, according to time; A) Cellular markers, B) MMPs and TIMPs, C) Growth factors.

that decreased to be minimal on day seven ($P=.042$). MMPs increased after coil occlusion and statistical differences between times were found for MMP-9, maximal on day 14 ($P=.043$) and MMP-14, increasing from days four to seven ($P=.002$) while TIMP-2 decreased with time after day four ($P=.064$) and TIMP-4 remained unchanged (figure 2B). MCP-1 was elevated at all times, while TGF- β increased to be maximal on day seven ($P=.219$) and PDGF-BB decreased on day seven ($P=.210$). There was no significant modification of Angiopoietins (figure 2C).

b) Genes associated with strategies to prevent recanalization compared to platinum coil occlusion

There were significant differences between groups in the expression of vWF on days four and seven ($P=.015$): arteries treated with radioactive coils or with denudation and coiling showed decreased vWF as compared to platinum coils (figure 3A).

Arteries treated with radioactive coils showed a significant effect of time on the expression of vWF, with a progressive decrease, minimal on day 14 ($P=.014$), and on the expression of SMA, minimal on day seven ($P=.015$).

There was a significant increase in the expression of CD14 on days four and seven when arteries treated by radioactive or non-radioactive coils, but not when arteries were denuded and coiled ($P=.002$) (figure 3B).

There was no significant difference in expression of MMPs (figure 3C) or TIMPs between groups.

There was decreased expression of MCP-1 with denudation and coiling as compared to platinum coils, but the difference was not significant ($P=.20$). As compared to arteries treated with platinum coils, arteries treated with radioactive coils or denudation and coiling showed lesser increased TGF- β 1 on day seven (figure 3D), and increased expression of PDGF-BB on day seven, but again differences between groups were not significant. Arteries treated by denudation without coil occlusion showed decreased expression of vWF and SMA, with a rapid recovery and increased expression by day seven ($P=.009$), a recovery not shown when arteries were simultaneously coiled.

Figure 3 Genes associated with recanalization or its inhibition. mRNA levels of vWF, CD14, MMP-9 and TGF- β 1 between the three treatment groups, are plotted as specimen/control artery ratio, according to time. *: $P=0.015$.

Discussion

Arterial recanalization after coil occlusion is a cellular process that occurs in a delayed fashion, within days, and as such it should be distinguished from thrombolysis^{3,4,12,13}. There is a constant pattern after coil occlusion of canine arteries: occlusion persists for six to seven days but is reliably followed by recanalization by 15 days when coils are non-radioactive³⁻⁵.

The pertinence of the recanalization phenomenon in this arterial occlusion model is supported by studies showing that its inhibition, through radiation or endothelial denudation, leads to better angiographic outcome in experimental aneurysm models that have a propensity to recur³⁻⁵. This model differs from the clinical situation where selective occlusion

of the aneurysm is attempted, while flow in the parent vessel is preserved. The model allows the study of the recanalization phenomenon independently from other variables, such as geometry, residual flows in residual necks and aneurysm size. Because 'treated' and 'control' arteries are compared in a paired fashion, biological variability may be decreased. There is a theoretical concern that treatment of one artery may affect results in another location. The beta radiation emitted from ³²P-coils penetrates only a few mm of tissues⁴. Because different locations were at least 10 cm apart one from the other, it is improbable that radiation emitted from one location affects another target artery. Finally, constant recanalization of arteries treated with platinum coils, irrespective of location or of groups suggests that a treat-

ment that prevents recanalization in one location does not preclude recanalization in the other.

We were mainly interested in the differential gene expression in arteries that recanalized or not. The arterial occlusion model provided a simple and reproducible model to study genes associated with recanalization or its inhibition without the variability intrinsic to aneurismal models. If recanalization of coiled arteries is pertinent to aneurysm recurrences, the search for associated mechanisms may provide future avenues to improve endovascular treatment without the potential drawbacks of radiation or mechanical denudation of aneurysms.

We concentrated our molecular investigation on the first two weeks because functional effects of recanalization were effective at two weeks in all cases, and because in the absence of recanalization (using denudation or radiation) during this early period, it did not occur later on when animals were followed for three months³.

Molecular patterns associated with platinum coil occlusion can be interpreted in the light of our initial hypothesis, combining gene expression involved in both neointima formation and angiogenesis. The most important finding was increased vWF mRNA levels, maximal at seven days, compatible with the early formation of endothelialized channels through the clot. This pattern was inhibited by both strategies designed to prevent recanalization (figure 2,3). Although platelets contain small amounts of vWF mRNA 14, increased expression persisted at seven days only in arteries treated with platinum coils (figure 3). The major source of vWF mRNA is endothelial; thus we propose that vWF was a marker of endothelial invasion essential to recanalization. We have shown that recanalization was associated with the formation of endothelial cell-lined longitudinal channels crossing the thrombotic occlusion^{3,4} (figure 1A). The evolution of SMA mRNA levels was more complex; we hypothesize an initial decrease caused by medial trauma or disuse related to absence of blood flow, compensated by progressive infiltration of myofibroblasts, maximal by 14 days. The fact that differences in vWF mRNA, but not SMA mRNA levels, were observed in the presence or the absence of recanalization, suggests that permanent occlusion obtained with radioactive coils, or denudation and coiling, is due to inhibition

of recanalization, rather than to stimulation of neointima formation.

The origin and nature of recanalizing and neointimal cells remains a subject of debate; they could come from migration of parietal cells or seeding of circulating cells.

The role of circulating bone marrow-derived cells has been emphasized in experiments where deficient organization and recanalization in uPA^{-/-} mice were rescued by infusion of wild type "progenitor" cells¹³. Bone marrow cells have a varying contribution to neointima formation, depending on animal models^{15,16} and various circulating cellular lineages may be involved in organization or recanalization^{12,16,17}. The fact that the original endothelial lining seems essential to recanalization is at first glance an argument against the role of circulating progenitor cells in this phenomenon, but perhaps some sort of cellular cross-talk or signal from endothelial cells, is necessary for recruitment and orderly adhesion of circulating cells¹⁸. Human CD 34+ cord blood cells can differentiate into cells that express both vWF and SMA¹⁹. The controversy regarding the role or even the identity of circulating cells in angiogenesis is underlined by reports proposing that they may be derived from bone marrow monocyte lineage cells^{20,21} and may be more active by secreting growth factors than by a direct cellular participation in the construction of the "new vessels"²². A role for monocytes drilling tunnels has recently been described in recanalization, an observation made in MCP-1 overexpressing mice¹⁸. In this experiment CD14 mRNA levels, a marker of monocyte-lineage cells were increased in arteries treated with both radioactive and non-radioactive coils. Local recruitment of CD14+ cells seems to require the presence of the endothelial lining perhaps through MCP-123, since CD14 was not increased when arteries were treated by denudation and coiling (P=.002). However, increased CD14 expression does not seem to discriminate arteries that recanalize from those that remain occluded (figure 3B).

Growth factor mRNA patterns were not significantly different but one may wonder if an important finding is missed because of the small number of observations.

The MMPs play a central role in degrading extracellular matrix, a necessary step for angiogenesis, which could also be important to thrombus organization and recanalization, at

least for MMP-9 (figure 2B). There was no significant difference however between coiled arteries that recanalize and those treated with ^{32}P coils or denudation (figure 3C).

The present work differs from our previous publication in murine carotid arteries in which recanalization was less reliable (found in only half of animals), and in which vWf was found to be decreased after radioactive or non-radioactive coil occlusion, perhaps from unavoidable endothelial injury during coil implantation in such a small animal model²⁴. It did not permit to identify mechanistic molecular events that would offer a comprehensive understanding of recanalization, in contrast to organization, after coil occlusion. We assumed that both methods to prevent recanalization would act through a common mechanism, but this assumption remains uncertain. Healing after coil occlusion involves two phenomena, recanalization and organization of thrombus. Recanalization, at the molecular level, translates into increased level of vWF mRNA. It is still unclear whether this observation reveals a cause or an effect of recanalization, or is merely epiphenomenal. Furthermore, it is unknown if it applies to human aneurysms. Strategies designed to prevent early seeding or migration of

endothelial or progenitor cells could promote permanent occlusion of aneurysms, but perhaps at the cost of increased risks of thromboembolic complications.

Potential weaknesses of this work include 1) that we may not have selected the appropriate genes potentially responsible for recanalization and 2) the small number of animals for each time point used for molecular analysis. It is also important to emphasize that RT-PCR gives no indication regarding biological activity.

Conclusions

Endothelial denudation and in situ beta radiation can prevent recanalization. At the level of a molecular language, recanalization translates into early increased of vWF mRNA levels. Strategies designed to prevent the formation of endothelialized channels may prevent recanalization.

Acknowledgments

This work was supported by the Canadian Institutes of Health Research and the Heart and Stroke Foundation of Canada.

References

- 1 Molyneux A, Kerr R et Al: International Subarachnoid Aneurysm Trial (ISAT) of neurosurgical clipping versus endovascular coiling in 2143 patients with ruptured intracranial aneurysms: A randomised trial. *Lancet* 360: 1267-1274, 2002.
- 2 Raymond J, Guilbert F et Al: Long-term angiographic recurrences after selective endovascular treatment of aneurysms with detachable coils. *Stroke* 34: 421-427, 2003.
- 3 Raymond J, Leblanc P et Al: In situ beta radiation to prevent recanalization after coil embolization of cerebral aneurysms. *Stroke* 33: 421-427, 2002.
- 4 Raymond J, Leblanc P et Al: Beta radiation and inhibition of recanalization after coil embolization of canine arteries and experimental aneurysms: How should radiation be delivered? *Stroke* 34: 1262-1268, 2003.
- 5 Raymond J, Guilbert F et Al: Role of the endothelial lining in persistence of residual lesions and growth of recurrences after endovascular treatment of experimental aneurysms. *Stroke* 35: 1471-1475, 2004.
- 6 Raymond J, Sauvageau E et Al: Role of the endothelial lining in persistence of residual lesions and growth of recurrences after endovascular treatment of experimental aneurysms. *Stroke* 33: 850-855, 2002.
- 7 Ribourtout E, Raymond J: Gene therapy and endovascular treatment of intracranial aneurysms. *Stroke* 35: 786-793, 2004.
- 8 Raymond J, Salazkin I et Al: Endovascular treatment of experimental wide neck aneurysms: Comparison of results using coils or cyanoacrylate with the assistance of an aneurysm neck bridge device. *Am J Neuroradiol* 23: 1710-1716, 2002.
- 9 Raymond J, Guilbert F, Roy D: Neck-bridge device for endovascular treatment of wide-neck bifurcation aneurysms: Initial experience. *Radiology* 221: 318-326, 2001.
- 10 Raymond J, Mounayer C et Al: Safety and effectiveness of radioactive coil embolization of aneurysms: effects of radiation on recanalization, clot organization, neointima formation or on surrounding nerves in experimental models. Submitted to *Stroke*, 2006.
- 11 Raymond J, Salazkin I et Al: High concentration ethylene-vinyl alcohol copolymer and endovascular treatment of experimental aneurysms: Feasibility of embolization without protection devices at the neck. *Am J Neuroradiol* 24: 1778-1784, 2003.
- 12 Wakefield TW, Linn MJ et Al: Neovascularization during venous thrombosis organization: A preliminary study. *J Vasc Surg* 30: 885-892, 1999.
- 13 Singh I, Burnand KG et Al: Failure of thrombus to resolve in urokinase-type plasminogen activator gene-knockout mice: Rescue by normal bone marrow-derived cells. *Circulation* 107: 869-875, 2003.
- 14 Rox JM, Bugert P et Al: Gene expression analysis in platelets from a single donor: Evaluation of a PCR-based amplification technique. *Clin Chem* 50: 2271-2278, 2004.
- 15 Tanaka K, Sata M et Al: Diverse contribution of bone marrow cells to neointimal hyperplasia after mechanical vascular injuries. *Circ Res* 93: 783-790, 2003.
- 16 Han CI, Campbell GR, Campbell JH: Circulating bone marrow cells can contribute to neointimal formation. *J Vasc Res* 38: 113-119, 2001.
- 17 Waltham M, Burnand KG et Al: Vascular endothelial growth factor enhances venous thrombus recanalisation and organisation. *Thromb Haemost* 89: 169-176, 2003.
- 18 Moldovan NI, Asahara T: Role of blood mononuclear cells in recanalization and vascularization of thrombi: Past, present, and future. *Trends Cardiovasc Med* 13: 265-269, 2003.
- 19 Lu X, Dunn J et Al: Smooth muscle alpha-actin expression in endothelial cells derived from CD34+ human cord blood cells. *Stem Cells Dev* 13: 521-527, 2004.
- 20 Fujiyama S, Amano K et Al: Bone marrow monocyte lineage cells adhere on injured endothelium in a monocyte chemoattractant protein-1-dependent manner and accelerate reendothelialization as endothelial progenitor cells. *Circ Res* 93: 980-989, 2003.
- 21 Nakul-Aquarone D, Bayle J, Frelin C: Coexpression of endothelial markers and CD14 by cytokine mobilized CD34+ cells under angiogenic stimulation. *Cardiovasc Res* 57: 816-823, 2003.
- 22 Rehman J, Li J et Al: Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 107: 1164-1169, 2003.
- 23 Humphries J, McGuinness CL et Al: Monocyte chemoattractant protein-1 (MCP-1) accelerates the organization and resolution of venous thrombi. *J Vasc Surg* 30: 894-899, 1999.
- 24 Raymond J, Lebel V et Al: Recanalization of arterial thrombus, and inhibition with beta-radiation in a new murine carotid occlusion model: mRNA expression of angiopoietins, metalloproteinases, and their inhibitors. *J Vasc Surg* 40: 1190-1198, 2004.

Jean Raymond, M.D.
 Interventional Neuroradiology
 Research Laboratory
 CHUM Research Centre
 Notre-Dame Hospital
 1560, Sherbrooke east, suite M-8203
 Montréal, Québec, Canada. H2L 4M1
 E-mail: dr_jean_raymond@hotmail.com