Concomitant Coiling Reduces Metalloproteinases Levels In Flow-Diverter Treated Aneurysms But Anti-Inflammatory Treatment Has No Effect

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Abstract

Background and purpose: Flow diverters (FD) can cause rare but devastating delayed aneurysm ruptures due to a potential implication of matrix metalloproteinases (MMPs). Concomitant coiling or anti-inflammatory medications have been proposed to prevent the risk of delayed ruptures. The aims of this study were to evaluate concomitant coiling and cyclosporine to regulate the MMPs expression in FD treated aneurysms.

Materials and methods: Elastase-induced aneurysms were created in 20 rabbits. Aneurysms were treated with 1) FD alone, 2) FD with concomitant coiling 3) FD and cyclosporine or 4) left untreated as controls. At sacrifice, MMPs levels were analyzed via zymography. Kruskal-Wallis one-way non-parametric ANOVA was performed for each enzyme. If significant results were observed for the Kruskal-Wallis test, pairwise group comparisons were performed using Dunn's test with Bonferroni multiple-testing correction.

Results: Significant differences were observed among groups for pro-MMP9 (p=.0337). Pairwise comparison demonstrated higher levels of pro-MMP9 with concomitant coiling compared to untreated aneurysms (p=.012), with higher though not significantly different levels of pro-MMP9 in FD with concomitant coiling versus FD alone. While not statistically significant, trends were noted regarding differences in active-MMP9 across groups with lower level of active-MMP9 with concomitant coiling compared to the other FD groups. No significant differences were observed for pro- or active-MMP2 across groups, nor for FD with cyclosporine compared to FD alone.

Conclusions: FD implantation increases the level of pro-MMP9 expression in aneurysms. Provocative trends regarding modulation of active-MMP9 expression with concomitant coiling suggests the need for larger, confirmatory preclinical studies. Anti-inflammatory treatment with cyclosporine appears to have minimal biological effect.

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Introduction

Flow diverters (FD) are now largely accepted as standard of care in treatment of selected aneurysms due to their high rates of angiographic occlusion and good clinical outcomes[1–3]. However, these devices may have rare but severe complications such as post-operative or delayed aneurysm rupture[4–8]. Despite the fact that numerous case series and case reports have reported on this complication, there continues to be controversy surrounding its origin with poor evidence regarding the risk factors and mechanisms of these hemorrhagic complications[4, 6, 8–12]. Prior studies have suggested a potential role of intra-aneurysmal thrombus in the pathophysiologic mechanism of aneurysm rupture[7].

As previously described in abdominal aortic aneurysms, leukocytes trapped in the intra luminal thrombus are a source of storage, release and activation of various proteases such as metalloproteinases (MMP2 and MMP9) and serine proteases. These have high proteolytic activity which could participate in the degradation of structural components of the arterial wall and lead to aneurysm rupture[7, 13–20]. MMPs are secreted as inactive proforms (pro-MMP) and are activated by protein cleavage by other proteinases (active-MMP)[20]. The over expression of activated type IV collagenases MMP9 and MMP2 in cerebral ruptured aneurysms[21] indicate that effective regulation of MMPs may result in improving clinical prognosis of cerebral aneurysms.

Some studies have already described the implication of MMPs in cerebral vascular diseases and aneurysms[22–29]. Previous studies have demonstrated a higher risk of rupture in giant aneurysms[2, 6, 7, 12] and have recommended that giant aneurysms are treated with concomitant coiling and flow diverter treatment in order to protect the dome of the aneurysm in an attempt to prevent delayed ruptures[4, 6, 11, 30, 31].

Cyclosporine A is an anti-inflammatory agent[32] widely used to prevent organ transplant rejection or treat autoimmune disorders[33]. Cyclosporine has already been tested for its effect on MMPs levels in various models and disorders[32–42]. In a study about abdominal aortic aneurysms, cyclosporine decreased MMP9 and stabilized expanding aortic arteries[38]. However, cyclosporine has not been tested in intracranial aneurysms to regulate the MMPs expression.

The aims of our study were to evaluate the effect of associated coiling and cyclosporine to regulate the MMPs expression in flow diverter treated aneurysms in a rabbit model.

METHODS

Aneurysm Creation and Treatment

The Institutional Animal Care and Use Committee approved all procedures before the initiation of this study. Some of the rabbits used in this study were originally employed as part of other investigations, where we investigated the gene expression between aneurysms treated with microcoils and flow diverters[43]. Elastase induced saccular aneurysms were created in 20 New Zealand White rabbits as previously described[44]. Three weeks after the aneurysm creation, rabbits were treated either with FD alone (n=5), FD with concomitant

coiling (n=6), FD and cyclosporine (n=5) or left untreated (n=4)[45, 46]. The rabbits treated with FD and cyclosporine were given 10 mg Cyclosporine A/kg body weight by oral gavage once daily for 4 weeks.

Rabbits were sacrificed 4 weeks after the treatment procedure. At the time of sacrifice, animals were deeply anesthetized. The animals were then euthanized with a lethal injection of pentobarbital. Aneurysms were immediately harvested, and frozen in liquid nitrogen and kept at −70°C until used[43].

MMPs Gelatin Zymography

Frozen samples were pulverized under liquid nitrogen and extracted in ice-cold lysis buffer (10 mmol/l sodium phosphate, pH 7.2, 150 mmol/l NaCl, 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, and 0.2% sodium azide). After centrifugation at 10,000 g for 20 min at 4°C, the protein concentration of the supernatant was determined (Pierce Biotechnology). Samples were resolved by nonreducing 10% SDS-PAGE through gels containing 0.1% gelatin (Bio-Rad). Gels were washed with 2.5% Triton X-100 for 1 h, then incubated for 24 h at 37°C in developing buffer (50 mmol/l Tris-HCl, pH 8.5, 5 mmol/l CaCl2 and 0.5 mmol/l ZnCl2). MMPs-2 and -9 are collagenases, which act on the gelatin (a partial collagen digest) in the gel and produce gelatinolytic zones. Gelatinolytic zones, representing the activities of MMPs, were visualized after staining the gels with 0.5% Coomassie blue R-250. The gelatinolytic zones of MMP-2 and MMP-9 were analyzed densitometrically using Image-J software and converted to quantifiable data in the number of pixels[47, 48]. The intensities of the gelatinolytic bands reflect the activity of corresponding MMP.

Statistics

Kruskal-Wallis one-way non-parametric ANOVA was performed for each enzyme (pro-MMP2, active-MMP2, pro-MMP9, and active-MMP9) for each of the four treatment groups. Kruskal-Wallis p-values were not corrected for multiple testing. Post-hoc pairwise group comparisons were performed using Dunn's test with Bonferroni multiple-testing correction[49]. Statistical analyses were performed in R (version 3.1.1; Vienna, Austria). Dunn's test was performed using R package dunn.test (version 1.2.4). A value of $\alpha=0.05$ was selected as the significance threshold.

RESULTS

Summary statistics are reported in Table 1 as medians and interquartile range (IQR). Figure 1 represents MMPs expression by aneurysm treatment with boxplots and individual data points.

In all treatment groups, levels of MMPs in treated aneurysms appeared higher than in the untreated group, with the exception of the FD with concomitant coiling group for active MMP-9; however, significant differences between groups were only found for pro-MMP9. MMP2 levels were similar throughout the three different FD treatment groups.

Compared to the untreated aneurysms, aneurysms treated with FD alone had higher levels of pro-MMP9 (2575, IQR: 697–4917; versus 428, IQR: 90–1941), active-MMP9 (2666, IQR: 1066–5291; versus 483, IQR: 251–1897), pro-MMP2 (4644, IQR: 2403–7693; versus 3250, IQR: 2950–6684) and active-MMP2 (3500, IQR: 2684–4476; versus 1294, IQR: 1143–1666) but those differences did not reach significant levels (Table 2).

In the group treated with FD and concomitant coiling the level of active-MMP9 was similar to the untreated group (496, IQR: 420–1734; versus 483, IQR: 251–1897). The level of pro-MMP9 was significantly higher in the FD with concomitant coiling group than in the untreated aneurysms group (9774, IQR: 7657–13562; versus 428, IQR: 90–1941; p value=0.012) (Table 2).

We did not observe any statistically significant difference or trends in difference when comparing the MMPs levels in aneurysms treated with FD + cyclosporine and FD alone.

DISCUSSION

This study demonstrates that treatment with FD affects MMPs levels in intracranial aneurysms. Specifically, aneurysms treated with FD and concomitant coiling demonstrate significant increased levels of pro-MMP9. In our current, relatively small study, we noted a trend toward decreased active-MMP9 with concomitant coiling compared to the other FD groups. Anti-inflammatory medications with cyclosporine did not significantly impact levels of MMPs. These findings suggest the need for larger, preclinical studies focused on MMP9 biology following treatment with FD.

By analogy to abdominal aneurysms, previous experimental and clinical studies have suggested that the intra-aneurysmal thrombus associated with FD could be a site of activation of MMPs and a potential cause of delayed ruptures[7, 13–19]. Furthermore, prior studies have suggested or demonstrated a higher risk of delayed ruptures after FD in giant aneurysms[2, 6, 7, 12]. Since giant aneurysms are generally more likely to have a larger intraluminal thrombus, it is probable that larger FD treated aneurysms have higher levels of MMPs expression. Based on our current findings, we believe that ongoing focus on MMP9 may provide important insights into FD-related complications.

The trend toward decreased active-MMP9 with concomitant coiling compared to other FD groups is of very high importance. Indeed, despite previous recommendations for concomitant coiling in aneurysms larger than 15 mm[4, 6, 11, 30, 31], no study described its effect on MMPs levels. Our study shows that the effect of concomitant coiling is not only related to a mechanical effect of the coils to protect the aneurysms dome but, at least in part, related to a biological effect on MMPs expression. It suggests that concomitant coiling may reduce the level of active-MMP9 expression in the FD treated aneurysms by blocking the activation of pro-MMP9 in its active form with accumulation of its inactive proform, which could be a potential solution to prevent delayed aneurysms ruptures after FD.

Anti-inflammatory and immunosuppressive drugs such as cyclosporine A have proved to be beneficial on MMPs levels in abdominal aneurysms[38, 50, 51]. However, in our study, the expression levels of MMP2 and MMP9 in the group treated with FD and cyclosporine were

comparable to aneurysms treated with FD alone with higher levels of active-MMP9. While it was hoped that cyclosporine could be used to control MMPs levels, it does not appear to have an effect on either MMP9 or MMP2. Further research could be done to explore the possibility of using other anti-inflammatory medications on MMPs expression. Additionally the role of pro-inflammatory mediators in aneurysm progression and rupture versus healing after treatment remains to be elucidated. Prior studies have demonstrated that MMPs and MCP-1 play key roles in formation and rupture with MCP-1 promoting MMP9, but post treatment expression also increases possibly due to aneurysm healing[29, 52, 53].

Limitations

Our study has several limitations. We used the rabbit elastase model which has histological, morphological, biological, and hemodynamic similarities to humans and is stable in time with no spontaneous thrombosis[54]. However, this model is neither a model of spontaneous rupture nor a model of delayed aneurysm rupture after FD and some biological aspects may differ when considering rupture-prone aneurysms. To explore these mechanisms, it would be of interest to analyze levels of MMPs in new models for active aneurysms with inflamed aneurysms wall or bio-active thrombus[55, 56]. Also, the reported cases of delayed ruptures after FD occurred mostly in large or giant aneurysms but the aneurysms used in this study were less than 20 mm. Some of our results did not reach significant differences but our ability to detect differences between groups was limited by the size of the treatment groups. Further studies should be done with larger groups. For the effect of concomitant coiling, we did not do any analysis of the impact of the packing density. Perhaps a denser coils packing would increase the effect of MMPs regulation. Different anti-inflammatory drug would maybe have different results but we decided to use cyclosporine because rabbits are known to be extremely sensitive to steroids[57–60]. Further, we analyzed only one dose for cyclosporine administration and its effect may be different with higher doses. Only one time point was studied following treatment of the aneurysm and protein expression may change over time. Finally, only two MMPs were studied and other MMPs isoforms may play important roles in delayed aneurysm rupture[61]. Transformation growth factor (TGF)-β is a key factor for MMP down-regulation. However, we did not measure the level of TGF-β in this study[38]. It may be possible that other proteolytic enzymes such as cathepsin or other pathways lead to delayed aneurysm rupture.

Conclusion

FD implantation increases the level of pro-MMP9 expression in aneurysms. Provocative trends regarding modulation of active-MMP9 expression with concomitant coiling suggests the need for larger, confirmatory preclinical studies. Anti-inflammatory treatment with cyclosporine appears to have minimal biological effect.

Abbreviations:

MMPs	Matrix Metalloproteinases
FD	Flow Diverter

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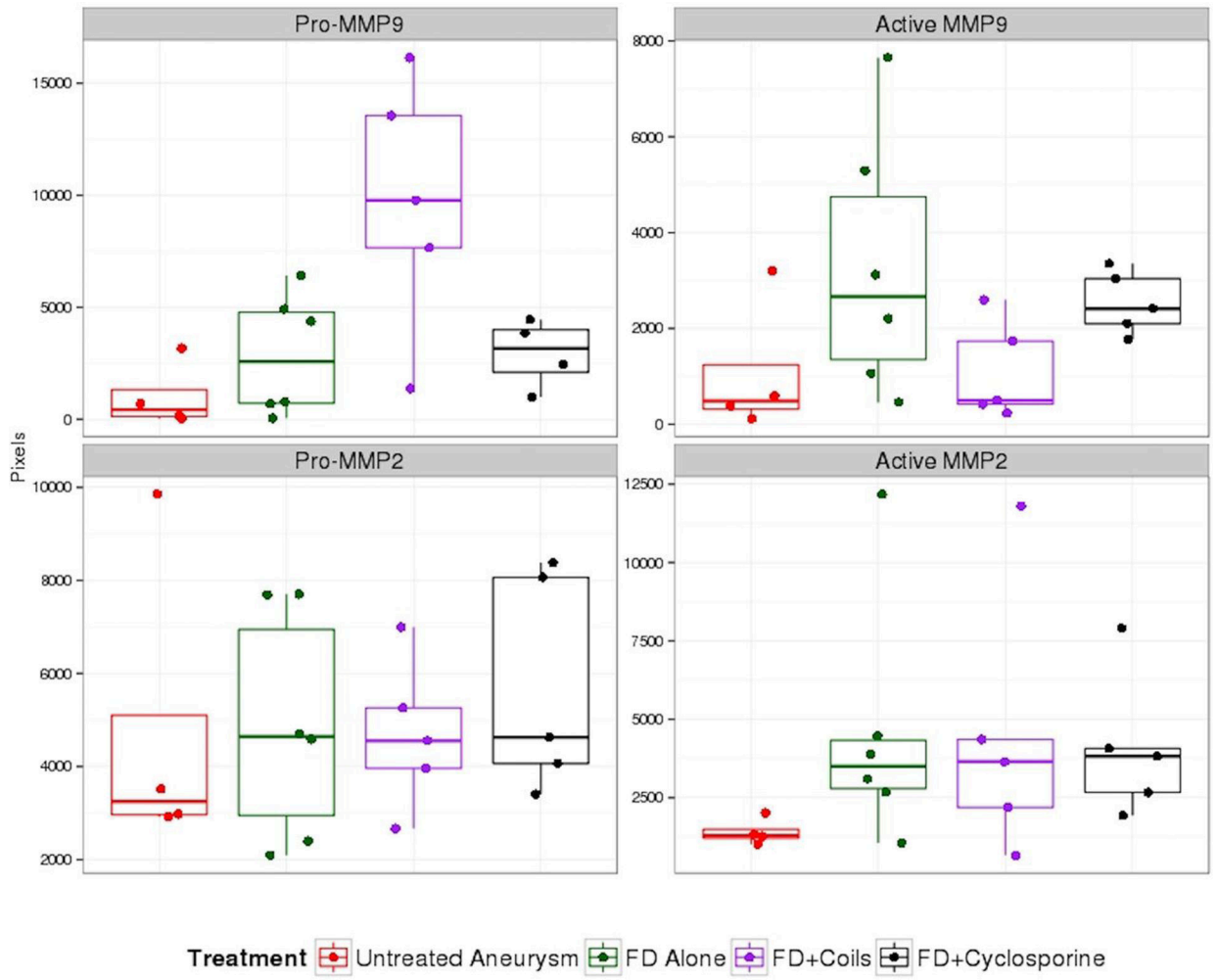
Figure 1:
Boxplot of MMPs expression by aneurysm treatment. The individual data points for each treatment are also shown. FD=flow diverter

Table 1:

Descriptive statistics for pixel counts by enzyme. Kruskal-Wallis tests.

	Untreated Aneurysm (N=4)	FD alone (N=6)	FD+ Coils (N=5)	FD+ Cyclosporine (N=5)[*]	p value
Pro-MMP9					0.0337
Median	428	2575	9774	3159	
IQR	90 – 1941	697 – 4917	7657 – 13562	1730 – 4150	
Active-MMP9					0.126
Median	483	2666	496	2410	
IQR	251 – 1897	1066 – 5291	420 – 1734	2099 – 3039	
Pro-MMP2					0.779
Median	3250	4644	4553	4628	
IQR	2950 – 6684	2403 – 7693	3962 – 5261	4066 – 8065	
Active-MMP2					0.152
Median	1294	3500	3653	3822	
IQR	1143 – 1666	2684 – 4476	2186 – 4360	2670 – 4068	

[*] Flow diverter + Cyclosporine Pro-MMP9 N=4

Table 2:

Post-hoc pairwise group comparison p-values for Pro-MMP9 using Dunn's method with Bonferroni correction.

	FD + Coils	FD alone	FD + Cyclosporine
FD alone	0.141	---	---
FD + Cyclosporine	0.414	1.00	---
Untreated Aneurysm	0.012	0.783	0.561