Chelation therapy: an alternative medicine or breakthrough cardiovascular treatment?

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Human blood vessels contain a diverse group of enzymes needed to speed up the rate of chemical reactions in our cells known as matrix metalloproteinases (MMPs). These require certain metals to operate, for example zinc and calcium. What is interesting about MMPs is that when they become uncontrolled, they contribute to a range of diseases including atherosclerosis and myocardial infarction (heart attack). This is because MMPs can cause the blood vessels to remodel themselves, fatty plaques in the vessel can become detached and perhaps spread to the brain or heart. To avoid this cells have a mechanism in place to stop MMPs going awry, namely, tissue inhibitors of metalloproteinases (TIMPs). TIMPs are small proteins that turn off MMPs. In the diseases mentioned, there is an imbalance in MMPs being active and TIMPs turning them off. This commentary looks at novel strategies that scientists have developed to prevent this imbalance such as chelation therapy, MMP inhibitors and compounds that cause our cells to make more TIMPs. Finally, this commentary will highlight the direction of future research into therapies and the likelihood of their use.

Metalloproteinases and MMPs: what are they and how are they regulated?

The use of enzymes in biological systems that utilise the characteristics of certain metals, commonly zinc, cobalt and manganese, to exert a catalytic effect are well understood (Schitler, 2019). Perhaps the most interesting class of these enzymes are the metalloproteinases which are used to break the peptide bond in protein chains. For example, pre-sequence protease (PreP) is used in degrading some mitochondrial proteins and the β-amyloid protein (Johnson *et al.*, 2006). Hence, it is unsurprising that a significant reduction in expression of PreP is seen in Alzheimer's disease, where β-amyloid deposits form pathologically significant plaques in the brain. PreP, also referred to as metalloproteinase-1, requires a Zn^{2+} ion to function and is, therefore, considered a metalloproteinase.

On a chemical level, the metal component of a metalloproteinase coordinates with three amino acid residues and a fourth coordination occurs with a water molecule. The water molecule binds fleetingly and is replaced by a target substrate amide bond (Hernick & Fierke, 2010). The importance of the metal is to polarise the carbonyl group of the target peptide which then allows the hydrolysis reaction to proceed.

The most significant subcategory of metalloproteinases is the matrix metalloproteinases (MMP) although adamalysins, serralysins and astacins also exist. MMPs contain zinc, but also depend on the presence of calcium (Mutluay-Tezvergil *et al.*, 2010). Their main role is to degrade extracellular membrane proteins, necessary for such processes as apoptosis, proliferation, migration and defence. Broadly speaking, there are six categories of MMP: collagenases, matrilysins, stromelysins membrane-type metalloproteinases, gelatinases and, finally, zinc- and calcium-dependent endopeptidases.

There are three mechanisms of action elucidated for MMPs: a base-catalysis reaction (Browner *et al.*, 1995), the Matthew acid-base reaction (Kester & Matthews, 1977) (proposing further use for water and the Zn^{2+} ion) and the Manzetti mechanism (Manzetti *et al.*, 2003). The latter showed Matthew's acid-base reaction was unlikely, and that histidine plays a more important role than zinc and water. Nevertheless, these hydrolysing mechanisms target the extracellular matrix, in particular, collagen and elastin fibres such as those found in the tunica media, adventitia and intima layers of the arterial wall (Xu & Shi, 2014). They are usually secreted from cells in an inactive zymogenic form.

A key regulator of MMPs are the TIMPs family of inhibitors, of which there are four (TIMP-1, TIMP-2, TIMP-3 and TIMP-4). TIMPs are biologically conserved endogenous proteins present in both primitive and higher eukaryotes (Murphy, 2011). Because of their essential role indicated by their prevalence in nature, it is unsurprising that any imbalance between MMP and TIMP activity can develop into a significant clinical pathology, e.g. cardiovascular disease.

Cardiovascular disease and MMPs

Recently, the pathological significance of certain MMPs has been observed in cardiovascular disease, as well as cancer metastasis, osteoarthritis and cirrhosis, as shown in Table 1. In this, MMPs seem to encourage and accelerate atherosclerotic lesion formation in the intima layer of vascular walls (see Figure 1). MMPs degrade various extracellular proteins to allow vascular smooth muscle cell (VSMC) migration to the aforementioned intima layer (Ravn & Falk, 1999).

MMPs become pathologically significant during vascular plaque remodelling. In this, a pre-existing plaque undergoes a change. This involves the growth and breakdown of collagen fibres in the arterial wall's intima layer, the latter is performed by MMPs. Thus, active atherosclerotic plaques are associated with far greater

MMP Biomarker	Pathology	Reference
Over-expression of TIMP-1, excess inhibition of MMP	Hepatic Cirrhosis	(Roeb, 2018)
MMP-2, 3 and 9 in synovial joint	Rheumatoid arthritis and Osteoarthritis	(Burrage *et al.*, 2006)
MMP-2, 9, 14 break-down of periostin	Myocardial Fibrosis and Heart Failure	(DeLeon-Pennell *et al.*, 2018)
Over-expression of MMP-2	Breast cancer metastasis	(Figueira *et al.*, 2009)
Over-expression of MMP-3, 7 and 9	Atherosclerosis and plaque instability	(Olejarz *et al.*, 2020)

Table 1. Various pathologies associated with digression from normal MMP/TIMP expression and regulation.

levels of MMPs, especially MMP-3 and MMP-9. Pro-remodelling factors are secreted by local macrophages which activate MMPs causing matrix degradation. Disequilibrium between active MMP and inactive TIMP increases the risk of plaque rupture and eventual cardiovascular events. (Liu *et al.*, 2006)

During active plaque remodelling the fibrous cap (composed of VSMCs) becomes weaker, leading to a potentially dangerous instability and eventual rupture. This can have downstream consequences, such as myocardial infarction (MI), stroke and other ischaemic conditions associated with microvascular plaque deposition. Furthermore, a genetic polymorphism within the promoter region of the genes coding MMP enzymes seems to dictate the susceptibility for cardiovascular disease, in particular coronary artery disease (Shalia *et al.*, 2010). As a result of this, the polymorphic promoter can act as a genetic biomarker for pathological significant cardiovascular disease.

MMPs and TIMPs as therapeutic targets for cardiovascular disease

Because MMPs depend on metal to function, removing this metal with a chelating agent could provide therapeutic potential. One such chelating agent is ethylene-diaminetetraacetic acid (EDTA), commonly used in the treatment of periodontal disease (Liu *et al.*, 2016).

To best appreciate the nature of EDTA as a chelation therapy, one must understand the complex chemistry of chelates. That being, a chelate is a molecule with the ability to remove metal from a source, in this case, the MMP enzyme. This is due to complex thermodynamics making the higher dentate ligand (EDTA) more capable of forming a metal complex than the original source of the metal (MMP) (see Figure 2). This is the so-called chelate effect (Vallet *et al.*, 2003).

The first reported use of EDTA to treat cardiovascular disease was reported by Clarke *et al.* in 1956, against angina pectoris and symptomatic coronary pathologies. This study demonstrated that angina was diminished in the majority of cases.

What does the future hold for MMP targeting?

EDTA and chelation therapy has not been approved for the treatment of MI and other cardiovascular diseases, although a large-scale study is currently being undertaken. TACT2 is a randomized, double blind controlled trial investigating EDTA and high-dose oral vitamins and minerals as a way to prevent recurrent cardiac events in diabetic patients with a prior MI and follows a decade long study which identified a significant effect of EDTA infusions on diabetic patients post-MI (Escolar *et al.*, 2014).

The current study, TACT2, aims to reach fruition by 2023 (Clinicaltrials.gov, NCT02733185). In particular, TACT2 aims to determine if chelation-based therapy increases the time to the first occurrence of adverse cardiovascular outcomes including hospitalization for unstable angina.

Besides EDTA it is also logical to assume that because TIMPs are endogenous inhibitors of MMPs the upregulation of TIMPs could serve as a therapeutic target. In fact, deficiency in certain TIMP subtypes, e.g. TIMP3, has been shown to precipitate pathological remodelling of the heart, cardiac fibrosis, abdominal aortic aneurysm and atherosclerosis, amongst other cardiovascular diseases (Fan & Kassiri, 2020).

Studies of TIMP infusion therapy in mice and rats has shown promising results. Studies have shown that reducing MMP activity in post-MI mice by administration of injections

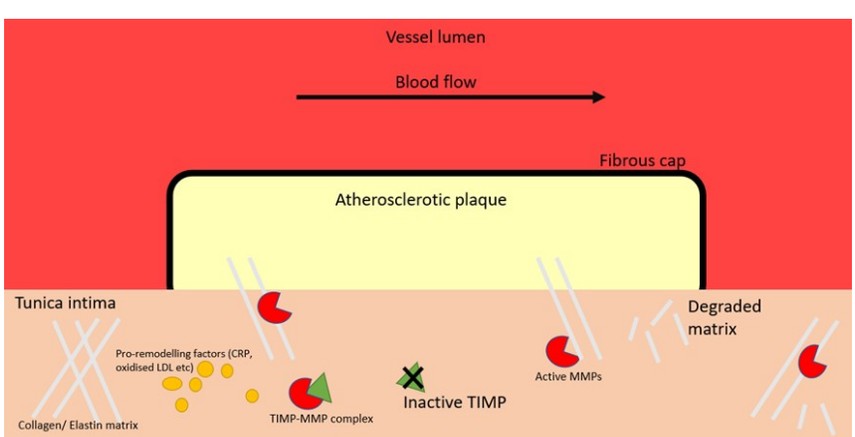

Figure 1. Vascular remodelling of the collagen/elastin matrix in the tunica intima .

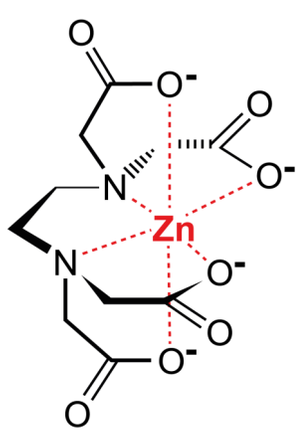

Figure 2. Molecular structure of the EDTA-zinc complex formed as a product of an MMP chelation reaction.

containing adenovirus' overexpressing TIMP3 into the peri-infarct area resulted in improved cardiac function after a single week (Takawale *et al.*, 2017). It is because of this that further research is being undertaken into the pharmacological implications for TIMPs as treatments for cardiovascular disease.

Conclusion

This commentary does not have the scope available to discuss the complete breadth of the effects of MMPs on the cardiovascular system. Greater information is available on a range of conditions due to excess MMP activity and metal deposition in the body, e.g. Ca^{2+} in the brain forming acervuli and MMP activity in tumour migration and osteoporosis (Vigh *et al.*, 1998).

Furthermore, when the TACT2 results are published, further information on the effects of EDTA chelation in post-MI diabetic patients will be available. We may even see enhanced use of chelation therapy, perhaps offered by the NHS, for cardiovascular pathology in the future. However, this still would depend on regulatory approval and cost-effectiveness.

Overall, the aim to use chelation therapy to inactivate metalloenzymes and treat cardiovascular disease remains controversial due to a lack of substantial, long-term effectiveness data. The fact that chelating agents remove metal from the body is well accepted within the scientific community, although its transition from alternative to conventional cardiovascular therapy is currently met with caution (Sultan *et al.*, 2017). Despite the use of chelation therapy within the NHS to treat heavy metal toxicity and thalassemia (Fulgenzi *et al.*, 2015), redeployment within a cardiovascular scenario is yet to be demonstrated as efficacious. Despite the vast understanding of metal biochemistry, there remain many unanswered questions-especially involving MMPs and how significant they really are to human pathology.

References

Browner, M. F., Smith, W. W. & Castelhano, A. L. 1995. Matrilysin-inhibitor complexes: common themes among metalloproteases. *Biochemistry*, 34(20), 6602-10.

Burrage, P. S., Mix, K. S. & Brinckerhoff, C. E. 2006. Matrix metalloproteinases: role in arthritis. *Frontiers in Bioscience*, 1(11), 529-43.

Clarke, C. N., Clarke, N. E. & Mosher, R. E. 1956. Treatment of angina pectoris with disodium ethylene diamine tetraacetic acid. *American Journal of Medical Sciences*, 232(6), 654-666.

ClinicalTrials.gov [Online] National Library of Medicine (US). 2016 April 11. Identifier NCT02733185, Trial to Assess Chelation Therapy 2 (TACT2). Available from: https://clinicaltrials.gov/ct2/show/NCT02733185.

DeLeon-Pennell, K. Y., Meschiari, C. A., Jung, M., *et al.* 2018. Matrix Metalloproteinases in Myocardial Infarction and Heart Failure. *Progress in Molecular Biology and Translational Science*, 147, 75-100.

Escolar, E., Lamas, G.A., Mark, D.B., *et al.* 2014. The effect of an EDTA-based chelation regimen on patients with diabetes mellitus and prior myocardial infarction in the Trial to Assess Chelation Therapy (TACT). *Circulation: Cardiovascular Quality and Outcomes*, 7(1), 15-24.

Fan, D. & Kassiri, Z. 2020. Biology of Tissue Inhibitor of Metalloproteinase 3 (TIMP3), and Its Therapeutic Implications in Cardiovascular Pathology. *Frontiers in Physiology*, 11(61), DOI: 10.3389/fphys.2020.00661.

Figueira, R. C. S., Gomes, L.R, Neto, J.S, *et al.* 2009. Correlation between MMPs and their inhibitors in breast cancer tumor tissue specimens and in cell lines with different metastatic potential. *BMC Cancer*, 9(20), DOI: 10.1186/1471-2407-9-20.

Fulgenzi, A., De Giuseppe, R., Bamonti, F., *et al.* 2015. Efficacy of chelation therapy to remove aluminium intoxication. *Journal of Inorganic Biochemistry*, 152, 214-8.

Hernick, M. & Fierke, C. 2010. Mechanisms of Metal-Dependent Hydrolases in Metabolism. *Comprehensive Natural Products II*, 8, 547-581.

Johnson, K. A., Bhushan, S., Ståhl, A., *et al.* 2006. The closed structure of presequence protease PreP forms a unique 10,000 Angstroms3 chamber for proteolysis. *Europe PMC*, 25(9), 1977-1986.

Kester, W. R. & Matthews, B. W. 1977. Crystallographic study of the binding of dipeptide inhibitors to thermolysin: implications for the mechanism of catalysis. *Biochemistry*, 16(11), 2506-2516.

Liu, P., Sun, M. & Sader, S. 2006. Matrix metalloproteinases in cardiovascular disease. *Canadian Journal of Cardiology*, 22, 25-30.

Liu, X., Mao, M. & Ma, T. 2016. The effect of EDTA root conditioning on periodontal surgery outcome: A meta-analysis. *Quintessence International*, 47(10), 833-841.

Manzetti, S., Herrington, A. C., van der Spoel, D. *et al.* 2003. Modeling of enzyme–substrate complexes for the metalloproteases MMP-3, ADAM-9 and ADAM-10. *Journal of Computer-Aided Molecular Design*, 17, 551-565.

Murphy, G. 2011. Tissue inhibitors of metalloproteinases. *Genome Biology*, 12(233), DOI: 10.1186/gb-2011-12-11-233.

Mutluay-Tezvergil, A., Agee, K.A., Hoshika, T., *et al.* 2010. The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices. *Dental Materials*, 26(11), 1059-1067.

Olejarz, W., Lacheta, D., Kubiak-Tomaszewska, G. 2020. Matrix Metalloproteinases as Biomarkers of Atherosclerotic Plaque Instability. *International Journal of Molecular Sciences*, 21(11), 3946, DOI: 10.3390/ijms21113946.

Ravn, H. B. & Falk, E. 1999. Histopathology of plaque rupture. *Cardiology Clinics,* 17(2), 263-270.

Roeb, E. 2018. Matrix metalloproteinases and liver fibrosis (translational aspects. *Matrix Biology*, 68-69, 463-473.

Schitler, D. 2019. Finding the right match. *Nature Reviews Chemistry*, 3 (130), DOI: 10.1038/s41570-019-0083-5.

Shalia, K. K., Shah V.K., Mashru, M.R., *et al.* 2010. Matrix metalloproteinase-3 (MMP-3) -1612 5A/6A promoter polymorphism in coronary artery disease in Indian population. Indian *Journal of Clinical Biochemistry*, 25(2), 133–140.

Sultan, S., Murarka, S., Jahangir, A., *et al.* 2017. Chelation therapy in cardiovascular disease: an update. *Expert Review of Clinical Pharmacology,* 10(8), 843-854.

Takawale, A., Zhang, P., Patel, V.B., *et al.* 2017. Tissue Inhibitor of Matrix Metalloproteinase-1 Promotes Myocardial Fibrosis by Mediating CD63-Integrin β1 Interaction. *Hypertension*, 69(6), 1092-1103.

Vallet, V., Wahlgren, U. & Grenthe, I. 2003. Chelate Effect and Thermodynamics of Metal Complex Formation in Solution: A Quantum Chemical Study. *Journal of the American Chemical Society*, 125(48), 14941-14950.

Vígh, B., Szél, A., Debreceni, K., *et al.* 1998. Comparative histology of pineal calcification. *Histology and Histopathology*, 13, 851-870.

Xu, J. & Shi, G. P. 2014. Vascular wall extracellular matrix proteins and vascular diseases. *Biochimica et Biophysica Acta*, 1842(11), 2106-2119.