

CATECHIN AND SULFATED POLYSACCHARIDE NUTRICEUTICALS INHIBIT MMP-2 (GELATINASE-A) OF FIBROBLASTS CULTURED FROM ABDOMINAL AORTIC ANEURYSMS



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Introduction

Matrix Metalloproteinases (MMPs) are involved in the cascade of proteolytic events that lead to aneurysmal dilation of the aorta. Invading inflammatory cells produce cytokines that induce resident mesenchymal cells to upregulate matrix metalloproteinases and result in increased destruction of extracellular matrix molecules [1-2]. Aneurysmal mesenchymal cells demonstrate increased MMP-2 transcription, protein levels and binding [3-5].

Matrix metalloproteinase-2 (MMP-2) is a 72 kilodalton (kDa) type IV collagenase thought to be the principle metalloproteinase in small aneurysms. It is produced by fibroblasts and is a member of a family of proteolytic enzymes that use metal for their catalytic mechanism. MMP-2 binds two zinc ions and four calcium ions per subunit and is responsible for cleavage of gelatin type I and collagen types IV, V, VII, X. The enzyme cleaves the collagen-like sequence Pro-Gln-Gly-Ile-Ala-Gly-Gln.

There is increased interest in new therapeutic strategies that aim at treating endothelial dysfunction. The levels of MMP-2 in early aneurysm development make it an ideal target. Initial clinical investigations using antibiotics, as inhibitors of MMPs have been disappointing. We tested extracts of green tea polyphenols and various forms of sea cucumber-derived sulfated polysaccharides against MMP-2 activity of fibroblasts cultured from human patients with abdominal aortic aneurysms (AAAs).

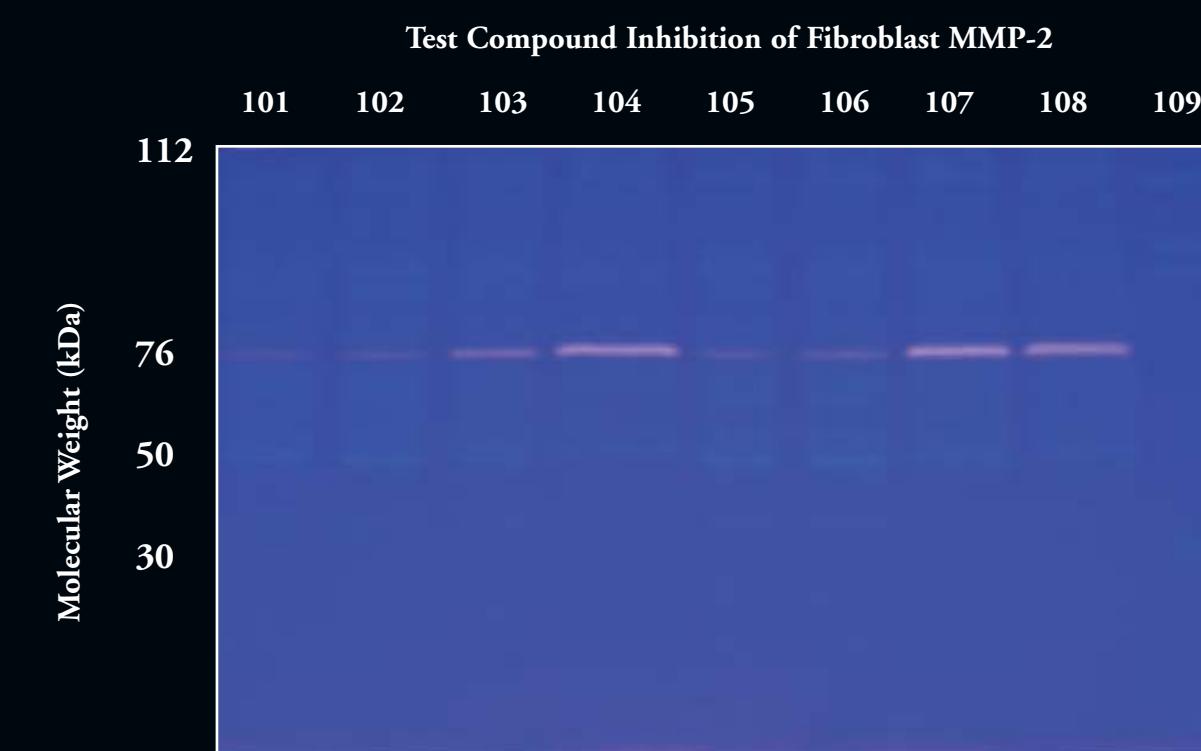
Methods

Properties of different test compounds against MMP-2 activity of aneurysmal fibroblast origin were examined on 10% polyacrylamide gels containing 1.0mg/ml of gelatin. Fibroblasts cultured from AAA specimens were lysed using the CellLytic-M lysis/extraction reagent (Sigma). The protein concentration of each supernatant was determined by the Bradford method using bovine serum albumin (Sigma) as a standard. The aortic fibroblast lysates were incubated alone (control) or with the test compounds of various concentrations for 1 hour on ice. After test compound incubation, equivalent amounts of proteins (30ug) were mixed with an equal volume of sample buffer (2% SDS, 125mM Tris-HCl, pH 6.8, 10% glycerol and 0.001% bromophenol blue) and then electrophoresed in Tris-glycine el. buffer (25mM Tris pH 8.3, 250mM Glycine, 0.1% SDS) under denatured but non-reduced conditions. After electrophoresis, the gels were incubated in 2% Triton X-100 for 30 min at room temperature to ensure SDS removal. Next, the gels were incubated at 37C overnight in development buffer (0.005M Tris-HCl pH7.5, 0.2M NaCl, 0.005M CaCl₂, and 0.02% Brij-35) (Bio-Rad). Gels were stained with 0.05% Coomassie blue R250 (Bio-Rad) for 30 min and destained twice in 40% methanol and 10% acetic acid for 20 min. Gelatinolytic activity was detected as unstained bands on a blue background. Quantity One (Bio-Rad Version 4.3.1) was used as a densitometer to quantitate test compound inhibitory ability.

Both green tea polyphenols and sea-cucumber derived sulfated polysaccharides partially inhibited MMP-2 activity (approximately 80%) at 100 ug/mL. Inhibition was increased when the two compounds were combined in a 1:1 ratio however dose-response curves indicated that this synergy was not straightforward.

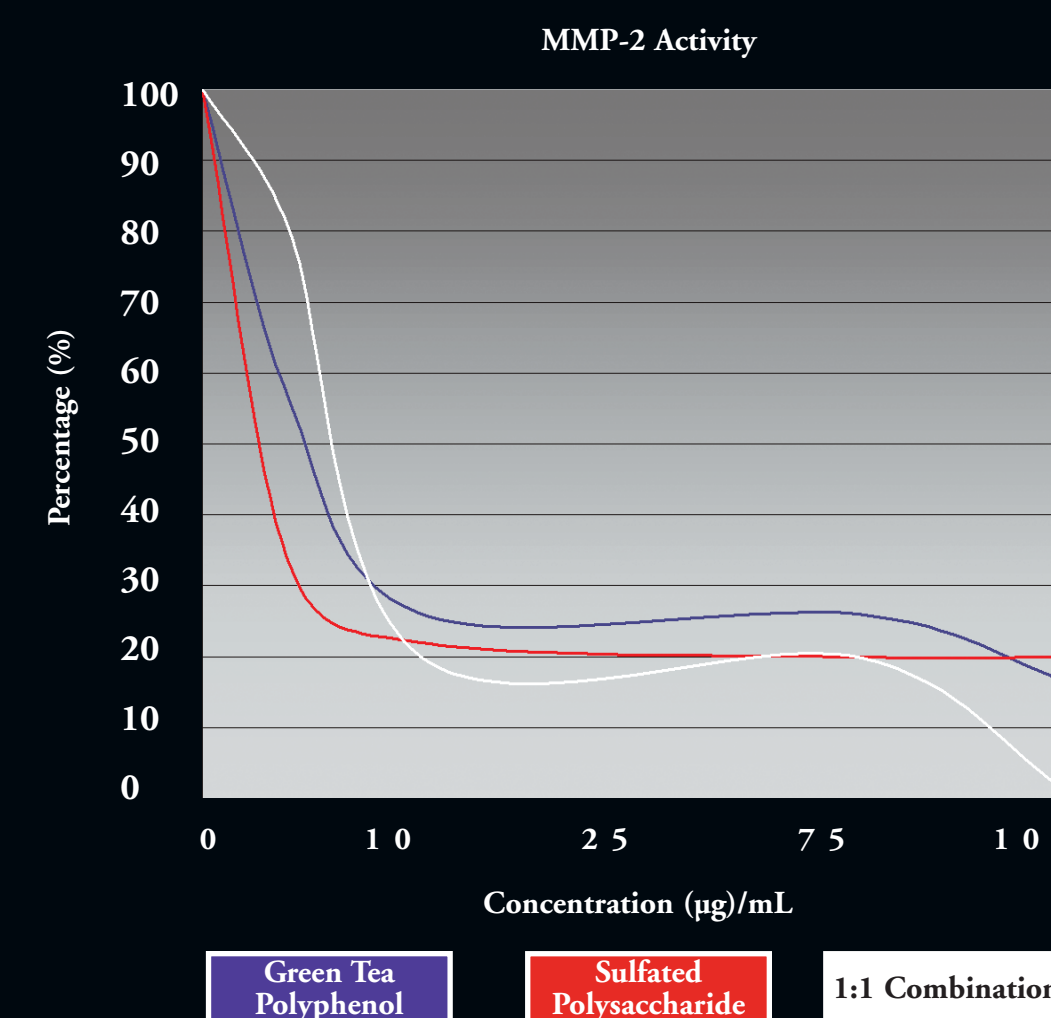
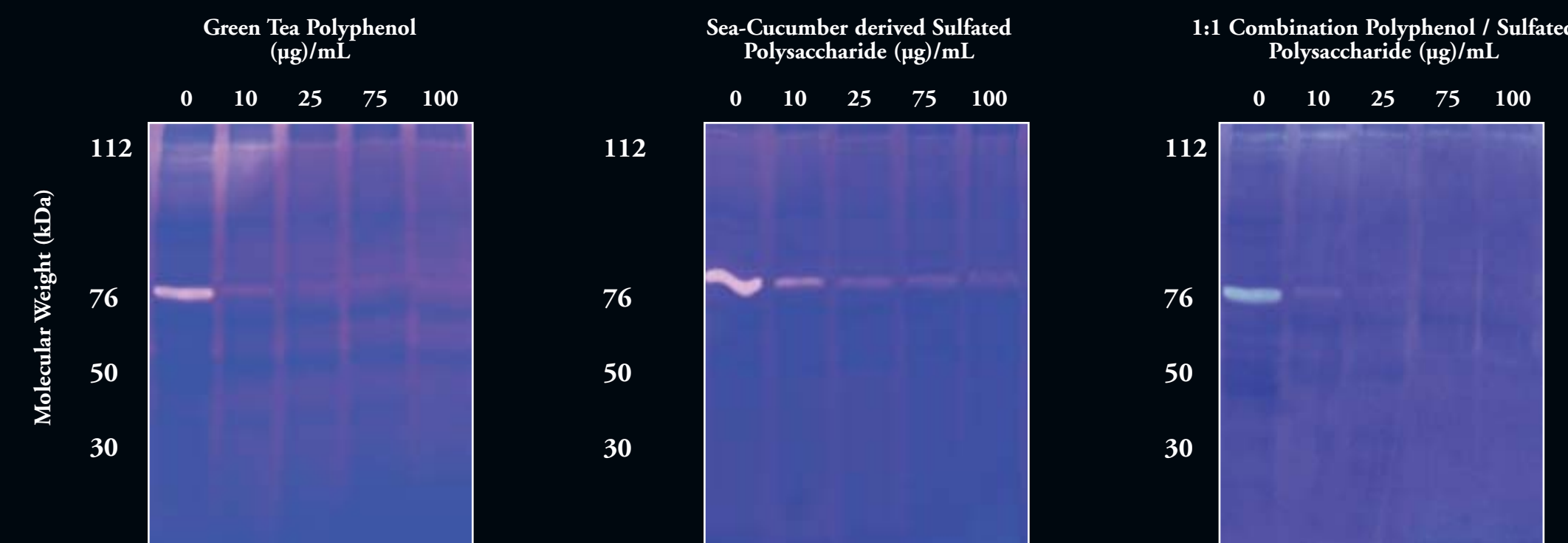
Additional compounds tested suggested patterns of inhibition that were variable and complex.

Results



Test compounds 101-105 correspond to various green tea polyphenols at 100 (ug)/mL. Compound 101 is shown below.

Test compounds 106-108 correspond to various sea-cucumber derived sulfated polysaccharides at 100 (ug)/mL. Compound 106 is shown below.



Discussion

The polysaccharides extracted from the sea-cucumber wall used in this experiment share a similar backbone structure to mammalian chondroitin sulfate but with a number of glucuronic acid residues displaying sulfated fucose branches. Fucosylated chondroitin sulfate has been previously shown to inhibit thrombin generation after stimulation by both contact-activated and thrombo-plastin-activated systems. The specific spatial array of these sulfated fucose branches is essential for the uniqueness of its anticoagulant action. [6]

Reports of the endothelial benefits of catechins and other dietary antioxidants are prevalent however mechanisms and definitive evidence are still ambiguous. Antioxidants may have an ability to inhibit the initiation of lipid peroxidation, [7] but reports are conflicting as to whether this is limited to an effect on a metal-ion dependent oxidizing system. Dietary antioxidant improvements in EDRF (endothelium-derived relaxing factor) action seem unrelated to any alteration in serum lipoproteins and appear independent to the extent of atherosclerosis. [8]

Our data suggests that the two families of compounds independently and together have an ability to inhibit fibroblast MMP-2 activity. Our experiments did not provide an explanation to the possible synergistic effects these compounds might share nor give adequate insight into an obvious mechanism. These effects were not additive at lower doses, nor specific to the ratios tested. Although elevated activities of metalloproteinases in aneurysmal disease is only one of many contributing factors, possible dietary supplementation of catechin containing compounds and sulfated polysaccharides may eventually be shown to have therapeutic benefit. Further experiments are needed.

Conclusion

Our data suggests that aneurysmal fibroblast MMP-2 activity can be inhibited by catechin containing compounds and sulfated polysaccharide nutraceuticals. Although our conclusions cannot yet be extrapolated to an *in vivo*, physiologic environment, there is a rationale for further experiments to determine possible therapeutic applications of natural MMP-2 inhibitors.

Bibliography

1. Freestone, T., et al., Inflammation and matrix metalloproteinases in the enlarging abdominal aortic aneurysm. *Arterioscler Thromb Vasc Biol*, 1995, 15(8): p. 1145-51.
2. McMillan, W.D., et al., In situ localization and quantification of seventy-two-kilodalton type IV collagenase in aneurysmal, occlusive, and normal aorta. *J Vasc Surg*, 1995, 22(3): p. 295-305.
3. Crowther, M., et al., Localization of matrix metalloproteinase 2 within the aneurysmal and normal aortic wall. *Br J Surg*, 2000, 87(10): p. 1391-400.
4. Longo, G.M., et al., Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. *J Clin Invest*, 2002, 110(5): p. 625-32.
5. Davis, V., et al., Matrix metalloproteinase-2 production and its binding to the matrix are increased in abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol*, 1998, 18(10): p. 1625-33.
6. Mourao, P.A., et al., Inactivation of thrombin by a fucosylated chondroitin sulfate from echinoderm. *Thromb Res*, 2001, 102(2): p. 167-76.
7. da Silva Porto, P.A., J.A. Laranjinha, and V.A. de Freitas, Antioxidant protection of low density lipoprotein by procyanidins: structure/activity relationships. *Biochem Pharmacol*, 2003, 66(6): p. 947-54.
8. Keaney, J.F., Jr., et al., Dietary antioxidants preserve endothelium-dependent vessel relaxation in cholesterol-fed rabbits. *Proc Natl Acad Sci U S A*, 1993, 90(24): p. 11880-4.