

Myriocin as an atherosclerosis inhibitor



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Targeting the sphingolipid pathway to treat atherosclerosis

Atherosclerosis is a major cause of cardiovascular disease and accounts for approximately 50% of all deaths in westernized countries [1]. Atherosclerosis develops as a consequence of multiple pathways that involve dysregulated cellular proliferation, lipid accumulation and inflammation [2,3]. Hypercholesterolemia is a major risk factor for atherosclerosis and it is clear that HMG-CoA reductase inhibitors (statins) effectively lower serum total and LDL-cholesterol (LDL-C), and reduce cardiovascular morbidity and mortality [4]. However, it is well known that a significant number of patients are either resistant to or intolerant to statins and, with recent guidelines for LDL-C levels to be reduced below 2 mM, it has been reported that fewer than 50% of patients treated with statins achieve their targets [5]. In order to address this problem, statin combination therapies are under investigation, for example, using the cholesterol-absorption inhibitor ezetimibe [5]. As an adjunct to statin therapy, strategies targeting proatherogenic pathways that are not primarily aimed at reducing cholesterol synthesis or absorption would be predicted to result in a two-pronged approach to treat atherosclerosis. As a case in point, therapeutic targeting of the sphingolipid biosynthetic pathway may represent a feasible approach to treat atherosclerosis [6,7].

Glycosphingolipids & atherosclerosis

Work from my own group [8,9] and two independent groups [10,11] has shown that the serine palmitoyl transferase (SPT) inhibitor myriocin is a potent inhibitor of atherosclerosis in *ApoE*^{-/-} mice. Myriocin inhibits the initial step in the sphingolipid biosynthetic pathway (Figure 1), so the precise mechanism(s) by which myriocin

exerts its potent antiatherogenic action is presently unknown. It is possible that myriocin may exert its effects by modulation of several lipids downstream of ceramide (such as glycosphingolipids [GSLs] and the bioactive lipids ceramide-1-phosphate [C1P] and sphingosine-1-phosphate [S1P]) and/or by altering the expression of antiatherogenic genes in the liver; and recent data indicate that *ApoA1* is one hepatic target [8,12].

We have previously emphasised the need to focus on selective inhibition of specific branches in the sphingolipid pathway in order to gain mechanistic insights into the most valuable therapeutic targets [7]. We demonstrated that atherosclerosis in *ApoE*^{-/-} mice is associated with increased plasma and lesion concentrations of GSLs [13]. More recently, we showed that the antiatherogenic activity of myriocin was associated with significant reductions in plasma GSL levels [8,9]. This led us to evaluate a selective GSL-synthesis inhibitor D-threo-1-ethylendioxyphenyl-2-palmitoylamino-3-pyrrolidino-propanol (EtDO-P4) as a potential inhibitor of atherosclerosis in *ApoE*^{-/-} mice. The data from this experiment indicated that 4 months of EtDO-P4 administration to *ApoE*^{-/-} mice (maintained on a high-fat diet) resulted in a 49% reduction ($p < 0.0001$) in plasma GSL levels compared with vehicle-treated mice, but this was not associated with a significant reduction in atherosclerosis [Glaros EN, Kim WS, Rye KA, Shayman JA, Garner B, Unpublished Data]. This implies that the antiatherogenic action of myriocin is also not likely to be based on reductions in GSL synthesis.

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Ceramide-1-phosphate & atherosclerosis

Myriocin could potentially regulate the synthesis of additional sphingolipids such as C1P. C1P is generated by ceramide phosphorylation catalyzed by ceramide kinase (Figure 1). It is

established that C1P is a potent activator of cytosolic phospholipase A₂α and, thus, a key regulator of macrophage cytokine-induced eicosanoid metabolism [14–18]. Macrophage foam cells present in atherosclerotic lesions secrete proinflammatory molecules that accelerate disease progression [2]. The products of macrophage eicosanoid metabolism, which include prostaglandins, leukotrienes and platelet-activating factors, are particularly important proinflammatory mediators (Figure 1). Identification of the major upstream regulators of macrophage eicosanoid metabolism and the design of drugs that inhibit such pathways is already recognized as a new avenue to treat atherosclerosis [19]. It remains possible that reductions in C1P generation achieved by myriocin could result in anti-inflammatory actions that inhibit the development of atherosclerosis in *Apoe*^{-/-} mice. However, direct evidence for this has so far not been provided.

Spingosine-1-phosphate & atherosclerosis

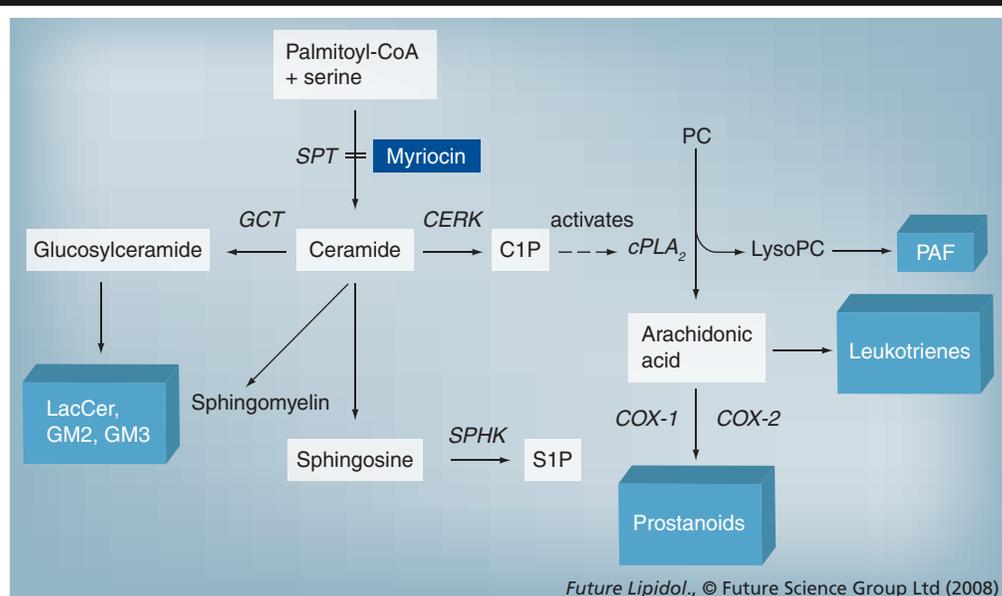
In contrast to C1P, which has not been studied *in vivo* as a therapeutic target for atherosclerosis, several studies have focused on the potential for the S1P analogue FTY720 to modulate atherosclerosis in both *Apoe*^{-/-} and *Ldlr*^{-/-} mice [20–23]. Two of the studies reported that FTY720

potently suppressed atherosclerosis in both the *Apoe*^{-/-} and *Ldlr*^{-/-} mouse models [20,21]; however, the third study indicated that atherosclerosis was not inhibited in *Apoe*^{-/-} mice treated with FTY720 and (in contrast to the other two studies) a marked hypercholesterolemia was reported in the drug-treated animals [22]. The reasons for this discrepancy are unclear, but it is worth noting that lesion data were reported only for the aortic root in the latter study and this site has been shown to be refractory to treatment in other work [9].

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It is thought that FTY720 is phosphorylated *in vivo* and exerts most of its antiatherogenic effects via interaction with S1P receptors (most likely S1P1 and S1P3), which results in an anti-inflammatory action [23]. At present, it is not clear how FTY720 inhibited lesion development in the two positive mouse studies. There were no consistent changes in either macrophage or CD3⁺ lymphocyte levels in lesions when the two studies were compared. Paradoxically, the potent

Figure 1. Sphingolipid pathway and inhibitor targets.



C1P: Ceramide-1-phosphate; CERK: Ceramide kinase; cPLA₂: Phospholipase A₂; GCT: Glucosylceramide transferase; LacCer: Lactosylceramide; LysoPC: Lysophosphatidylcholine; PAF: Platelet-activating factor; PC: Phosphatidylcholine; S1P: Spingosine-1-phosphate; SPHK: Spingosine kinase; SPT: Serine palmitoyl transferase.

antiatherogenic action of myriocin is associated with decreased plasma levels of S1P in the order of 73–81% [11].

Although one interpretation of these data could be that the antiatherogenic effect of myriocin could not be related to S1P production, our recent studies indicating that the liver is an important site of myriocin action [8] imply that there may be beneficial responses resulting from the reduced hepatic S1P levels that are predicted to result from myriocin treatment. It is well known that sphingolipids are important mediators of intracellular signaling pathways [24–26], and we are currently investigating hepatic signaling pathways that could potentially link the antiatherogenic action of myriocin with S1P and C1P.

Myriocin as a new therapeutic for the treatment of atherosclerosis?

At present, it appears unlikely that myriocin will be useful clinically. There are several reasons for this. As already noted, myriocin inhibits SPT, which blocks the first step in sphingolipid synthesis, and this is likely to impact on many members of the sphingolipid family that have important roles in signaling and regulation of cell growth, differentiation, apoptosis and

proliferation [27]. In addition, myriocin is an immunosuppressant (some data indicate it to be up to 100-fold more potent than cyclosporin [28]), which would preclude its long-term use as a treatment for cardiovascular disease in humans.

'At present, it appears unlikely that myriocin will be useful clinically.'

Myriocin has, however, provided an important tool that has allowed us to begin to investigate which specific members of the sphingolipid family may be selectively targeted to treat atherosclerosis. It appears quite possible that detailed investigations of the antiatherogenic actions of myriocin will reveal therapeutic targets that can be more selectively regulated in the future.

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The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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