PublisherInfo		
PublisherName	:	BioMed Central
PublisherLocation	:	London
PublisherImprintName	:	BioMed Central

MMP-3 targeted ribozymes - therapeutics for arthritis?

ArticleInfo		
ArticleID	:	155
ArticleDOI	:	10.1186/ar-2000-66841
ArticleCitationID	:	66841
ArticleSequenceNumber	:	112
ArticleCategory	:	Paper Report
ArticleFirstPage	:	1
ArticleLastPage	:	4
ArticleHistory	:	RegistrationDate : 2000–9–13 OnlineDate : 2000–9–13
ArticleCopyright	:	Current Science Ltd2000
ArticleGrants	:	
ArticleContext	:	130753311

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Keywords

Cartilage, MMP-3, proteoglycan, ribozymes, stromelysin

Context

Ribozymes are catalytic RNA molecules, they occur naturally but can also be synthesised to target specific mRNA molecules. In this study a panel of synthetic ribozymes containing the hammerhead motif were targeted against MMP-3 mRNA and were assessed for activity *in vitro* and in a cartilage explant model. Assess activity of MMP-3 targeted ribozymes using *in vitro* assays and the comparison with *in vivo* findings.

Significant findings

IL-1 stimulation of fibroblasts *in vitro*resulted in a 5-10 fold increase in MMP-3 expression after 18 h. Ribozymes that inhibited MMP-3 expression in this assay were shown to target sites in the 5'UTR (21), coding region (1049, 1363 and 1366) and 3'UTR (1489) of MMP-3 mRNA, whilst control ribozymes were inactive. The specificity of ribozyme action was determined by parallel measurement of IL-6 secretion, which was not altered. A ribozyme targeting site 1366 in the coding region displayed dose dependent inhibition of MMP-3 expression with an IC50 of ~240 nM and a similar efficacy was demonstrated against MMP-3 expressed from human synovial fibroblasts from osteoarthritis patients.

In the cartilage explant model labelled ribozymes were readily taken up into the tissue. Ribozyme 1366 reduced the level of MMP-3 mRNA extracted from cartilage explants but did not inhibit IL-1 induced proteoglycan release; however, IL-1 mediated cartilage degradation was completely inhibited by the broad spectrum MMP inhibitor Batimastat and by the transcription inhibitor actinomycin D.

Ribozyme uptake *in vivo* following intra-articular injection was evident in synovial fibroblasts but minimal penetration was observed in the cartilage. Importantly, both cell compartments are reported sources of MMP-3 following IL-1 activation.

Comments

This paper is best read with the earlier *in vivo* paper at hand as it contains the details of the ribozyme sequences and ribozyme activity *in vivo* (see additional information). The ability to use synthetic ribozymes to selectively inhibit the expression of metalloprotease (MMP)-3 (stromelysin) both *in vitro* and *in vivo*is impressive. Whilst it is disappointing that no inhibition of proteoglycan degradation was observed, this is explained by the fact that eliminating MMP-3 from the MMP cascade was not sufficient to give substantial benefits. The potential of ribozyme technology is impressive as it can be used to selectively target any gene product associated with a disease. Targeting mediators that initiate inflammatory events such as interleukin (IL)-1 or TNF-a with synthetic ribozymes may prove an effective local therapy in rhuematoid arthritis (RA).

Methods

Ribozymes used in this study contain a binding arm (7-8 nucleotides), which anneal to the MMP-3 message and an invariant core sequence (22 nucleotides) required for catalytic activity. Control ribozymes included inactive ribozymes that contained two mutations in the core, which eliminated cleavage activity and scrambled ribozymes in which the arm sequence is scrambled, to eliminate binding to the target sequence. Ribozymes are named according to their position of cleavage in the human MMP-3 sequence, and are rendered nuclease resistant through the presence of phosphioate linkages. In total, 24 ribozymes targeting cleavage sites in human MMP-3 were assayed for activity *in vitro*. Ribozymes complexed with lipofectamine were incubated with human foreskin fibroblasts (HS-27) for 3 h prior to stimulation of cells with IL-1. Supernatants were collected after 18 h and both MMP-3 and IL-6 levels measured by ELISA.

A cartilage explant model was used to assess ribozyme inhibition of MMP-3 and to determine the role of MMP-3 in IL-1 induced cartilage catabolism. Cartilage was obtained from rabbit knee joints, and slices stabilised in culture were stimulated with IL-1. Ribozyme activity was determined by measurement of MMP-3 mRNA levels extracted from explant tissue. Effects on cartilage catabolism were determined by the release of proteoglycan into the culture medium compared with the amount remaining in the cartilage.

Uptake of ribozymes into tissue was assessed in the explant culture by addition of labelled ribozyme to the media for a given time. *In vivo* uptake was assessed in healthy rabbit knee joints following intra-articular injection of ribozymes.

Additional information

Flory CM, Pavco PA, Jarvis TC, Lesch ME, Wincott FE, Beigelman L, Hunt III SW, Schrier DJ: **Nuclease-resistant ribozymes decrease stromelysin mRNA levels in rabbit synovium following exogenous delivery to the knee joint.** *Proc Natl Acad Sci USA* 1996, **93**:754-758 (PubMed abstract).

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