INHIBITION OF INTERLEUKIN-1-STIMULATED MATRIX METALLOPROTEINASE EXPRESSION IN TENDON CELLS BY THE GREEN TEA COMPOUND EPIGALLOCATECHIN GALLATE ESTER

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INTRODUCTION

The medicinal benefits of green tea (*Camellia sinensis*) have been attributed to a family of bioavailable polyphenols, of which epigallocatechin gallate (EGCG) is both the most abundant and one of the most potent in its antioxidant and biological activities (1). Recent work using an *in vitro* model of cartilage degradation has shown that EGCG inhibits collagen and aggrecan breakdown (2). However, EGCG only partially inhibited the activity of the collagenases matrix metalloproteinase (MMP)-1 and MMP-13 at concentrations two orders of magnitude higher than those inhibiting aggrecanases (3), suggesting that the inhibition (by EGCG) of collagen breakdown may instead be due to an effect on enzyme expression or activation.

Since tendon problems have been experienced by patients treated with broad-spectrum metalloproteinase inhibitors (4), it was of interest to examine whether EGCG would affect the expression of collagenases by human tendon cells. The aims of this study were to assess the effects of EGCG and its non-esterified counterpart (EGC), on the expression of mRNA encoding matrix metalloproteinases (MMP) in control and interleukin (IL)-1-stimulated human tendon-derived cells.

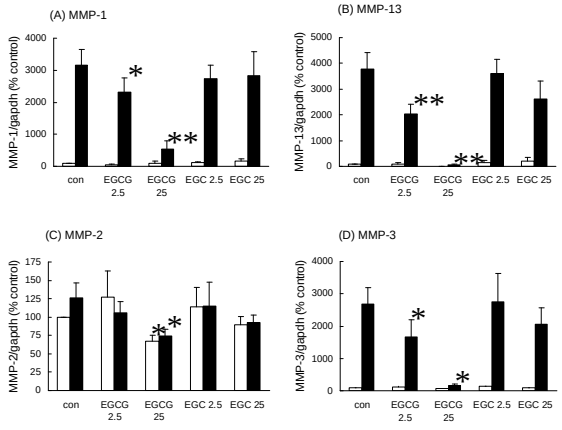
METHODS

Tendon specimens from three patients were obtained from tissue discarded during surgery for chronic Achilles tendinopathy, with informed patient consent and local ethical committee approval. Cells isolated by outgrowth from the separate tendon explants were used between passages 4 and 10. Cells were seeded at 10^5 /well in 6-well plates and were incubated for 3 days before the experiment. They were rinsed with serum-free DMEM containing insulin, transferrin and selenium, and were then given 2 ml of the same medium with or without EGCG or EGC. After 18 h, IL-1β (1 ng/ml: approx 60 pM) was added to stimulate the cells. Control cultures had equivalent additions of medium.

After incubation for a further 24 h, the cells were rinsed with balanced salts solution and solubilised in TRI-Reagent (1 ml/well). RNA was isolated from the extracts by phenol-chloroform phase separation followed by precipitation with isopropanol and ethanol. RNA was assayed for GAPDH, MMP-1, -2, -3 and -13 using semi-quantitative RT-PCR in a GeneAmp 5700 (Applied Biosystems, Warrington, UK).

The results were taken from three independent experiments, one from each of the three separate tendon isolates. In order to compare across experiments, the normalised expression of each gene in control cells was defined as 100%. Significant reduction of mRNA expression by EGCG or EGC relative to control pretreatment was assessed by paired *t*-test.

RESULTS



Tendon cells were given control pretreatment (con) or pretreated with EGC or EGCG at 2.5 or 25 μM, and then incubated without IL-1β (open columns) or with IL-1β (filled columns) for 24 hours. RNA was assayed for MMP mRNA normalised to GAPDH and expressed relative to controls. Mean ± SEM from three experiments. P < 0.05, ** P < 0.01.*

Tendon cells expressed each of the MMP mRNA species examined. Differences between the Ct values in the optimised realtime semi-quantitative RT-PCR assays indicated that in unstimulated tendon cells MMP-2 mRNA was expressed at levels at least 100-fold higher than the other metalloproteinase mRNA species. Of the collagenases, MMP-1 mRNA was expressed at more than 100-fold higher levels than MMP-13 mRNA.

Incubation of tendon cells with IL-1β resulted in an increased expression of MMP-1, MMP-3 and MMP-13 mRNA (typically 30-fold), while MMP-2 mRNA was not substantially affected.

EGCG significantly reduced the stimulation by IL1 - by 20-30% at 2.5μM and more than 80% at 25μM, and had a smaller effect on MMP-2 mRNA expression.

In all experiments EGC was at least 10-fold less potent than EGCG.

DISCUSSION

The biochemical effects of green tea may include a combination of actions of EGCG against proteinase expression and activity. EGCG has previously been shown to have a potent and direct inhibition of aggrecanase enzymic activity, which might account for the reduction in aggrecan breakdown in the cartilage degradation model (2). By contrast, the inhibition by EGCG of collagen breakdown in that system did not appear to be attributable to direct inhibition of collagenase activity (3). A number of studies have focussed on the effects of EGCG on the activation of MMP-2 by MT1-MMP, and in some of those studies effects were noted on gene expression (5).

In this study, we have shown that EGCG reduces the IL-1-stimulated expression of some matrix metalloproteinase mRNA species by human tendon cells in culture. In preliminary experiments, we have found similar effects (of EGCG) in both human and bovine chondrocyte cultures (not shown); this could provide a mechanism for the effects of EGCG observed on collagen breakdown in the cartilage model (2).
We conclude that effects on MMP expression may be relevant to the anti-arthritic or anti-catabolic actions attributed to green tea.

Large increases in MMP mRNA expression induced by inflammatory cytokines such as IL-1 have been attributed to the activation of transcription factors such as AP-1 and NFkappaB. EGCG has been shown to disrupt proteasome function, preventing the destruction of the inhibitory subunit IkappaB and hence preventing the activation of NFkappaB, which could explain the observed inhibition of MMP expression (6).

However, although we have observed the expected loss of IkappaB following the addition of IL-1β to the tendon cells, we have not detected any effect of EGCG (on IkappaB) in these cells under the conditions we have used (ANC and VA Curry, unpublished data). Further studies will be required to unravel the mechanism of the EGCG effects on IL1-stimulated MMP gene expression.

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