Nitric oxide-mediated invasion in Barrett’s high-grade dysplasia and adenocarcinoma

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Nitric oxide (NO) has been shown to induce double strand DNA breaks in Barrett’s oesophagus (BO) and in other cancers has a role in invasion. The specific aims of this study were to investigate whether NO can induce invasion in cells representative of different stages of Barrett’s progression and to determine possible underlying mechanisms. Physiological concentrations of NO that mimic luminal production of NO from dietary sources enhanced invasion in cell lines from high-grade dysplasia (GihTERT) and oesophageal adenocarcinoma (FLO) but not a non-dysplastic Barrett’s cell line (QtHERT). Real-time reverse transcription–polymerase chain reaction revealed that NO induced expression of matrix metalloproteinase (MMP)-1, -3, -7, -9 and -10 and tissue inhibitor of metalloproteinase (TIMP)-1, -2 and -3 in these cell lines. Furthermore, ex vivo treatment of Barrett’s biopsy samples with NO induced increases in MMP-1 and TIMP-1 expression, suggesting that NO enhances invasion through deregulating MMP and TIMP expression in epithelial cells. In keeping with these findings, microarray analysis and immunohistochemistry performed on biopsy samples showed enhanced expression of MMP-1, -3, -7 and -10 and TIMP-1 in the progression from non-dysplastic BO to adenocarcinoma, although this could not be directly attributed to the effect of NO. Thus, NO may play a role in Barrett’s carcinogenesis through deregulating MMP and TIMP expression to enhance invasive potential.

Introduction

The incidence of oesophageal adenocarcinoma (AC) has increased at an alarming rate in the past few decades (1). Although the exact aetiopathogenesis of AC is poorly understood, the rapid rate of increase suggests that environmental factors are probably involved. AC is known to arise from Barrett’s oesophagus (BO), which results from the metaplastic conversion of the stratified squamous epithelium to a columnar-lined intestinal-like epithelium (2). It is well accepted that chronic reflux is important in the development of BO (3,4), although the exact mechanisms remain unclear. It is becoming evident that components of the refluxate may also play a role in the progression from BO to AC. Both acid and bile have been shown to affect proliferation and differentiation (5–8). Acid can also inhibit apoptosis via activation of the mitogen-activated protein kinase pathways (7) and down-regulation of the caspase cascade (9), whereas acidified bile salt cocktail can activate the interleukin-6/signal transducer and activators of transcription 3 anti-apoptotic pathway (10).

There is increasing evidence to suggest that nitric oxide (NO) and other reactive nitrogen species are candidate factors involved in the development of AC. Endogenous inducible NO synthase is commonly up-regulated and activated under inflammatory conditions, including oesophagitis, as well as in BO and AC (11,12). However, a much more potent source of NO in the distal oesophagus in patients with reflux occurs through the reaction between salivary nitrite and refluxed acid in the presence of ascorbate (13,14). Nitrite is reduced to nitrous acid and a number of nitrosating species by acid, which in the absence of excess ascorbate can also lead to the production of potentially carcinogenic N-nitroso compounds (14,15). This normally occurs at the gastro-oesophageal junction, where saliva first encounters an acidic environment but has been shown to shift proximally into the oesophagus in patients with BO (13,16). Significantly, NO produced in the lumen is able to rapidly diffuse across epithelial membranes where it or its reactive derivatives are potentially able to exert carcinogenic effects through interactions with cellular substrates, including DNA and proteins (17–19).

NO is known to have a number of pathological effects in cancer in addition to its normal role as a signalling molecule. Depending on the context, NO can play multifaceted and contradictory roles in carcinogenesis and tumour progression, having both tumouricidal cytotoxic and cytostatic effects in addition to tumour-promoting effects, such as angiogenesis, gene regulation and DNA damage (20). The exact effect of NO seems to depend on dose and the kinetics of NO exposure, concentration of reactive intermediates, as well as the cell type (21,22). Recently, we modelled luminal production of physiological concentrations of NO in vitro and demonstrated that it is able to cause DNA double strand breaks without affecting cell survival in BO and AC cell lines, suggesting that NO could be responsible for the accumulation of genetic abnormalities in the development of AC (19). Indeed, NO has been implicated in epithelial carcinogenesis and tumour progression, including multiple cancer types of the gastrointestinal tract (23,24), and has been shown to be mutagenic in human cells and in mice (25,26).

In this study, we have investigated a role for luminal concentrations of NO in promoting invasion in AC in vitro. There is significant evidence from other cancers that indicates an important role for NO in metastasis, particularly in the process of invasion. NO has been shown to enhance invasiveness in colorectal AC cell lines (27) and in mammary tumours in both humans and mice (28–30). In a murine model, NO was able to promote tumour growth and metastasis by enhancing tumour cell migration, invasiveness and angiogenesis (29). The mechanism involved sequential activation of NO synthase, guanylate cyclase and mitogen-activated protein kinase (31). These pathways have also been shown to be involved in up-regulation of matrix metalloproteinase (MMP) expression (32–34), a family of enzymes known to be crucial in the process of extracellular matrix remodelling and invasion. For this study, we utilized an in vitro trans-well assay to investigate the effect of NO, at concentrations found in the lumen of the oesophagus, on the invasive activity of BO and AC cell lines. Furthermore, we examined the effect of NO exposure on the expression of members of the MMP and tissue inhibitor of metalloproteinases (TIMP) families.

Materials and methods

Cell lines

The oesophageal AC cell line, FLO (35) (gift from D. Beer, University of Michigan, MI, USA), was maintained in Dulbecco’s modified Eagle’s medium (Invitrogen, Paisley, UK) supplemented with 10% fetal calf serum (FCS; HyClone UK Ltd, Northumberland, UK) and 100 U/ml penicillin/streptomycin (Invitrogen). QtHERT and GihTERT cell lines, derived from BO with no dysplasia and high-grade dysplasia (HGD), respectively (36) (gift from P. Rabinovitch, University of Washington, Seattle, WA, USA), were maintained in MCDB-135 medium (Sigma–Aldrich, Dorset, UK) containing 5% FCS, 100 U/ml penicillin/streptomycin, 0.25 μM analog (Invitrogen). 5 μg/ml insulin–transferrin (Invitrogen), 4 mM sodium selenite (Invitrogen), 10 μM cholera toxin (Quadtech Diagnostics, Epsom, UK), 20 ng/ml epidermal growth factor (Sigma–Aldrich) and 0.4 μg/ml hydrocortisone (Sigma–Aldrich). Cells were grown in a humidified environment at 37°C and 5% CO2.
NO donor
Cells were treated with known concentrations of NO in the form of the NO donor MAHMA NONOate, 6-(2-Hydroxy-1-methyl-2-nitrosohydradizino)-N-methyl-1-hexanamine (Axxora, Nottingham, UK), dissolved in 0.1 N NaOH, pH 10.5. This donor has a half-life of ~3 min at pH 7.4 and was selected to mimic a burst of NO production similar to what would be expected to occur in the lumen of the oesophagus when acid reacts with salivary nitrite during a reflux episode (13,14).

In vitro invasion
Invasion assays were performed using a two-chamber assay system in 24-well tissue culture plates containing cell culture inserts with a pore size of 8 μm (BD Biosciences, Oxford, UK). Cell culture inserts were coated with MatrigelTM (BD Biosciences) diluted in serum-free medium (FLO cells) or low serum medium (0.5% FCS, GibiTERT and QHTERT cells) at a concentration of 0.25 mg/ml. Fifty thousand cells in serum-free medium (FLO) or 0.5% serum medium (GibiTERT and QHTERT) were added to inserts. To induce invasion, a serum gradient was created by adding medium containing 10% FCS to the bottom of each well. MAHMA NONOate (5–500 μM) or an equivalent volume of 0.1 M NaOH (control) was added to the cells in the insert. Invasion was assessed after 24 h by fixing and staining insert membranes with methanol and haematoxylin, respectively. For each membrane, the number of cells that had invaded was counted in eight randomly selected fields using an eyepiece graticule at ×100 magnification. Experiments were performed in duplicate on four separate occasions and results averaged.

SYBR-green real-time reverse transcription–polymerase chain reaction
Cells were treated with 50 μM MAHMA NONOate for 1, 3, 6 or 22 h. Control treatments were performed with an equivalent volume of 0.1 N NaOH for 22 h. RNA was extracted using TRIZol® (Invitrogen) according to the manufacturer’s instructions. RNA extractions were performed in triplicate on three separate occasions and triplicate RNA samples were pooled to give three pools of RNA for each time point.

For each sample, 2 μg of total RNA was reverse transcribed with Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI) according to the manufacturer’s instructions. cDNA was synthesized in a total volume of 20 μl using 1 μl of total RNA, 100 nM each of forward and reverse primer in a total volume of 20 μl. The PCR protocol consisted of 40 cycles of 30 s at 95°C, 1 min at 60°C and 1 min at 72°C. Relative transcript abundance was calculated using the formula relative transcript abundance = 10ΔCt, where ΔCt is defined as Ct(sample)–Ct(control) and Ct is the average cycle number at which the fluorescence passes through a defined threshold. The results were expressed as the fold difference compared with control.

Human tissue samples
All human specimens used in this study (ex vivo NO stimulation and microarray analysis) were collected from patients with BO (with presence of intestinal metaplasia) and oesophageal AC from Addenbrooke’s Hospital, Cambridge. Ethical approval was obtained from Local Research Ethics Committees prior to collection for each site and patients were individually consented.

Ex vivo stimulation with NO donor
Paired biopsy specimens were collected from nine patients from within areas of BO. None of the patients had histological evidence of dysplasia. Biopsies were maintained in Medium199 (BioSera Ltd, Ringmer, UK) in a humidified environment at 37°C and 5% CO2 for a maximum of 3 h. Paired specimens from the same patient were treated with MAHMA NONOate (150 μM) or an equivalent volume of 0.1 N NaOH (control) for 1 (n = 5) or 3 (n = 7) h, with an additional treatment with MAHMA NONOate (150 μM) at 30 min. RNA was extracted using TRIZol® according to the manufacturer’s instructions and SYBR-green RT–PCR carried out as for cell lines.

Immunostaining
Embedded paraffin specimens from intestinal metaplasia (n = 16), dysplasia (n = 17) and AC (n = 21) were deparaffinized in xylene then rehydrated in ethanol and then in water. Antigen retrieval was performed with R and D Systems Europe Ltd retrieval reagent (Abingdon, UK), followed by staining for MMP-1 and TIMP-1 (both from R and D systems) using the Dako Envision Kit (Dakooytimation Ltd, Ely, UK) following manufacturer’s instructions. The slides were then counter stained with haematoxylin. The stromal and epithelial compartments were scored separately. The stromal staining was scored by intensity ranging from weak (1) to medium (2) and strong (3). For MMP-1, the extent (scored 1 for 1–25% cells stained, 2 for 26–50%, 3 for 51–75% and 4 for 76–100%) and intensity was assessed for the epithelial compartment. For TIMP-1, the size of the clumps of cells staining positive was assessed (score of 1 for a single cell per field, 2 for small clump of two to three cells or more than two single cells per field and 3 for large clumps of cells). Antibodies were extensively validated, positive and negative controls were used and staining was reviewed by an expert upper gastrointestinal pathologist to ensure specificity of the staining.

Microarray analysis
Existing gene expression profiling data of messenger RNA extracted from serial sections of fresh frozen tissue specimens [BO without dysplasia (n = 21), BO with low-grade dysplasia (n = 17), BO with HGD (n = 13) and oesophageal AC (n = 8)] was interrogated to determine expression changes of MMPs and TIMPs across the disease spectrum. This microarray study of whole tissue sections was performed in parallel with gene expression microarray analysis of microdissected stromal tissue from the same specimens and the procedure is described in detail elsewhere (37). An additional serial section from each specimen was haematoxylin and eosin stained and assessed by two expert gastrointestinal pathologists and a consensus histopathological diagnosis formed. Data analysis was performed with the Rosetta Resolver gene expression analysis software (version 6.0; Rosetta Biosoftware, Seattle, WA) and MATLAB software (version 7.0.4; Mathworks, Natick, MA).

Statistics
Statistical analysis was performed using analysis of variance and Student’s t-test. The Mann–Whitney test was used to analyse the difference between treated and untreated biopsies at a given time point. The Jonkheere–Terpstra test was used to analyse the trend of MMP and TIMP expression with progression from BO to AC.

Results
NO enhances invasion in HGD and AC cells lines in vitro
We investigated the ability of physiological concentrations of NO to modulate the invasion of oesophageal cell lines using a NO donor,

Table I. SYBR-green real-time reverse transcription–polymerase chain reaction primer sequences

<table>
<thead>
<tr>
<th>Target</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
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<tbody>
<tr>
<td>GAPDH</td>
<td>5'-CACACGCTCCAGATCAGTCAGC-3'</td>
<td>5'-ATGGACTGTGTCATGATGTC-3'</td>
</tr>
<tr>
<td>MMP-1</td>
<td>5'-GGACTTTGTCGAGAAATCCCT-3'</td>
<td>5'-CTTTCAAGCCCAAAAGATTCC-3'</td>
</tr>
<tr>
<td>MMP-2</td>
<td>5'-ACCCAGATACCTCGAAGGAC-3'</td>
<td>5'-CCAATGTGCTTATGTCATCT-3'</td>
</tr>
<tr>
<td>MMP-3</td>
<td>5'-TCTGAAAGTCTGGGAGAAGG-3'</td>
<td>5'-AACCTCTCATCTTCTTAACTG-3'</td>
</tr>
<tr>
<td>MMP-7</td>
<td>5'-GAAATATGTCAGAGGACCCCG-3'</td>
<td>5'-ATATGATACAGGCTTATGATGGT-3'</td>
</tr>
<tr>
<td>MMP-9</td>
<td>5'-CTGGGTAAGAGTCTCTGAC-3'</td>
<td>5'-CAACCTGATCCTGATGATGATGATG-3'</td>
</tr>
<tr>
<td>MMP-10</td>
<td>5'-TGCAGTTAAAGGACATGGAAG-3'</td>
<td>5'-GTGAATATCTCTCAATAAAGGATG-3'</td>
</tr>
<tr>
<td>MMP-14</td>
<td>5'-TCTCCTTTCAAGGAGGACCAAG-3'</td>
<td>5'-TCAAATCTCTGGATTGCGG-3'</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>5'-AAATCCGACCTCGTCATAC-3'</td>
<td>5'-GGACCTCTTTCATACCTTTGTG-3'</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>5'-TGAAGAGGAAAGTGCATGCTTG-3'</td>
<td>5'-GCCCCCTTGACATCTTTATGTC-3'</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>5'-TGTAACCTGCAGATCAAGTTCC-3'</td>
<td>5'-CAGGGTAAACCCTAACAGTGAG-3'</td>
</tr>
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MAHMO NONOate, in an in vitro system. NO increased the invasion of both GihTERT cells and FLO cells through an artificial basement membrane (Figure 1). An example of the porous membrane from the cell inserts that was used for scoring invasion of FLO cells is shown (Figure 1A). For HGD cells (GihTERT), invasion was significantly increased at all concentrations of NO (Figure 1B, \( P < 0.0001 \)). The fold increase in invasion of NO treated compared with untreated cells ranged from 1.3 (5 \( \mu M \), \( P < 0.001 \)) to 1.6 (100 \( \mu M \), \( P < 0.001 \)) but was not concentration dependent. Similarly, invasion was significantly increased (\( P < 0.0001 \)) in AC cells (FLO) at concentrations of NO \( \geq 25 \mu M \) but not at the lowest concentration (Figure 1B). The fold increase in invasion of NO treated compared with untreated cells ranged from 1.4 (25 \( \mu M \), \( P < 0.001 \)) to 1.7 (500 \( \mu M \), \( P < 0.0001 \)). In contrast, NO did not alter the ability of non-dysplastic QhTERT cells to invade at the doses investigated (Figure 1B).

**NO alters the expression of genes known to be involved in invasion**

MMPs and TIMPs are known to be important in regulating invasive activity of cancer cells. Therefore, we used real-time RT–PCR to examine the effect of NO on the expression of a panel of MMPs (MMP-1, -2, -3, -7, -9, -10 and -14) and TIMPs (TIMP-1, -2 and -3) in oesophageal cell lines 1, 3, 6 and 22 h after the addition of 50 \( \mu M \) of MAHMA NONOate.

In FLO cells (Figure 2A), the most frequent trend for MMP gene expression was for a rapid induction at 1 h after addition of NO and a gradual decrease to basal expression levels by 22 h. NO-induced gene expression reached significance at 1, 3 and 6 h for MMP-1 (5.7-fold, \( P < 0.0001 \)), -7 (3.1-fold, \( P < 0.02 \), respectively) and at 1 h for MMP-9 (3.5-fold, \( P < 0.001 \)). In contrast, expression of MMP-14 was significantly down-regulated by NO at 1 and 6 h (0.2-fold, \( P < 0.01 \) and 0.3-fold, \( P < 0.02 \), respectively) in FLO cells. Expression of TIMPs in FLO cells was also regulated by NO, with TIMP-1 being significantly up-regulated at 1 and 3 h (2.1-fold, \( P < 0.001 \) and 1.7-fold, \( P < 0.01 \), respectively). In contrast to FLO and GihTERT cells, non-dysplastic QhTERT cells did not show any significant increases in the expression of MMPs in response to NO (Figure 2C) but instead had significantly decreased expression of MMP-2 and MMP-7 at 22 h after the addition of NO (0.6-fold, \( P < 0.03 \) and 0.7-fold, \( P < 0.02 \), respectively). In addition, there was a small but significant decrease in the expression of TIMP-1 at 6 h (0.7-fold, \( P < 0.05 \)).

**NO alters MMP-1 in human samples**

MMP-1, -3, -9 and TIMP-1 were the most consistently altered species in response to NO treatment in dysplastic and AC cells lines. Their expression level was assessed 1 and 3 h after the addition of 150 \( \mu M \) of MAHMA NONOate to ex vivo cultured biopsies. One hour after stimulation, the expression of the MMPs and TIMP-1 was not altered compared with a control biopsy from the same patient treated with NaOH. However, at 3 h, NO induced a significant increase in MMP-1 and TIMP-1 (Figure 3, \( P < 0.03 \) and \( P < 0.001 \), respectively).

**Expression of MMPs and TIMPs are deregulated in the progression from BO to AC**

In order to determine the physiological relevance of the MMPs and TIMPs, their expression across the Barrett’s metaplasia, dysplasia and carcinoma sequence was determined. Interrogation of gene expression array data demonstrated a general trend for increased MMP and TIMP expression in the progression from BO to AC, which reached significance for MMP-3 (\( P = 0.003 \)), -7 (\( P < 0.001 \)), -10 (\( P = 0.001 \)) and TIMP-1 (\( P = 0.005 \)) (Figure 4). Expression of MMP-3 and MMP-7 was increased by 4- to 6-fold and MMP-10 and TIMP-1 were increased 2- to 3-fold from BO to AC.

Expression of MMP-1 and TIMP-1 at the protein level was performed using immunohistochemistry in paraffin-embedded sections (Figure 5). No difference was observed in the stromal intensity or number of epithelial cells staining for TIMP-1 across disease states (data not shown). For MMP-1, the extent of epithelial cells staining with MMP-1 was statistically increased with progression to cancer (Figure 5, \( P > 0.001 \)).

**Discussion**

We recently showed that physiological concentrations of NO could cause non-lethal double-stranded DNA breaks in oesophageal cell lines and primary BO cells treated ex vivo (19), signifying that generation of NO in the lumen of the oesophagus may play a role in the accumulation of genetic abnormalities in the development of AC. In this study, we demonstrated that NO might play a role in up-regulating invasion via up-regulation of MMPs and TIMPs. Using an in vitro
N.J. Clemons et al.

system that models the invasion of cells through a basement membrane, we showed that physiological concentrations of NO enhanced the invasiveness of HGD (GihTERT) and AC (FLO) cell lines. This is preliminary evidence that NO may play a further role in Barrett’s carcinogenesis in addition to its DNA damaging effect. However, NO did enhance the invasive ability of non-dysplastic QhTERT cells, although NO did not affect invasion. Furthermore, in other studies, increased TIMP-1 expression in non-small-cell lung carcinomas correlated with cancer aggressiveness. In this study, expression of TIMP-1 was significantly increased in both GihTERT and FLO cells and in Barrett’s tissue following incubation with NO. Coutard et al. (46) have shown in studies of cerebral aneurysms in rats that increased TIMP-1 expression is associated with increased neuronal NO synthase expression. Although this form of NO synthase is not expressed in oesophageal epithelium, this supports the hypothesis that increased levels of NO in the oesophagus could increase the expression of TIMP-1.

As expected from their role in MMP inhibition, TIMPs largely have the opposite effect from MMPs on tumourigenesis. Deregulation of the normal balance between MMPs and TIMPs can give rise to changes in tumour activity as well as defining the overall proteolytic potential of the tumour (45). In this study, expression of TIMP-1 was significantly increased in both GihTERT and FLO cells and in Barrett’s tissue following incubation with NO. Coutard et al. (46) have shown in studies of cerebral aneurysms in rats that increased TIMP-1 expression is associated with increased neuronal NO synthase expression. Although this form of NO synthase is not expressed in oesophageal epithelium, this supports the hypothesis that increased levels of NO in the oesophagus could increase the expression of TIMP-1.

MMP-9 is principally inhibited by TIMP-1 (47), thus the simultaneous up-regulation of these two genes is seemingly contradictory in terms of promoting invasion. However, work by Simi et al. (47) showed that simultaneous over-expression of both of these genes in non-small-cell lung carcinomas correlated with cancer aggressiveness. Furthermore, in other studies, increased TIMP-1 expression in laryngeal carcinoma has been significantly correlated with lymph node metastasis (48) and with the metastatic spread of squamous cell carcinomas of the head and neck (49), implicating a role for TIMP-1 in the process of metastasis. Thus, it is important to recognize that it may be too simplistic to interpret phenotypic changes in terms of individual genes rather than networks of genes.

Interestingly, the expression of MMPs was generally seen to increase across the disease progression from non-dysplastic BO to AC (Figure 4). This correlates with what is observed in many tumours and appears to define the overall proteolytic potential of the tumour (45). Furthermore, the extent of epithelial MMP-1 protein expression was significantly increased with progression to cancer in human tissue samples. Although we cannot directly attribute this to exposure to NO, it is suggestive given that NO is able to induce MMP-1 expression in Barrett’s tissue.

Others have evaluated expression of several MMPs in oesophageal cancer. Expression of MMP-1 correlates with poor prognosis in oesophageal squamous carcinomas and ACs (50). A study by Salmela et al. (51) looked at expression in BO, AC and lymph node metastasis. MMP-7 and MMP-12 were already up-regulated in intestinal metaplasia and overall, MMP-7 appeared to be the primary MMP expressed by malignant cells in AC. In addition, TIMP-1 and TIMP-3 were differentially regulated during transformation (51).

In contrast, none of the MMPs or TIMPs was up-regulated by NO in non-dysplastic Barrett’s (QhTERT) cells nor did it affect invasion. However, NO did induce expression of another target gene, COX-2,
in these cells indicating that NO was having a biological effect (data not shown). This suggests that NO may only exert these effects in later stages of the metaplasia–dysplasia–carcinoma sequence and may indicate that further molecular genetic changes are required in the development of HGD for this to occur. Given that NO has been demonstrated to act through the cyclic guanosine monophosphate/mitogen-activated protein kinase pathway to up-regulate MMPs (32–34), it is possible that alterations to this pathway during the progression to HGD are responsible. A recent randomized phase II trial has examined the effect of the MMP inhibitor prinomastat or placebo in combination with preoperative chemoradiotherapy in patients with oesophageal AC (52). However, the study was small (seven in each arm) and was discontinued due to unexpected thromboembolic complications in both arms of the study and therefore, no conclusions could be made regarding the effect of preoperative MMP inhibition on outcome. Several other trials have looked at inhibition of MMPs in cancer, generally using non-specific inhibitors of MMPs (53). However, although preclinical trials have shown some efficacy, the initial results of clinical trials are disappointing. Part of the problem may be due to the fact that MMPs show functional redundancy (54) and therefore targeting the pathway upstream of the production of MMPs may be more appropriate.

In summary, our study showed that NO enhances invasion in oesophageal AC through modulation of MMP and TIMP expression and thus may be involved in promoting the progression of dysplastic

Fig. 3. Effect of NO on ex vivo expression of MMP-1, -3, -9 and TIMP-1. Data are expressed as the difference in Ct values between treated and untreated, with both normalized to glyceraldehyde 3-phosphate dehydrogenase, and plotted as a boxplot. A reference line is included at y = 0 representative of no change in expression between treated and untreated samples. (The circles represent outliers; asterisk represents P < 0.03 and double asterisks represent P < 0.001).

Fig. 4. Expression of MMPs and TIMPs in the progression from BO to AC. Expression of each target is calculated as the log10 ratio of the mean light intensity of the probes, normalized to Human Universal Reference RNA. Mean values are graphed and tabulated for BO without dysplasia (BO), low-grade dysplasia (LGD), HGD and oesophageal adenocarcinoma (AC).

Fig. 5. Expression of MMP-1 in the progression from BO (A) to dysplasia, Dys (B) to AC (C). Cumulative data are shown in panel D (double asterisks represent P < 0.001).
lesions in BO to invasive carcinoma. Given that BO and AC present as inflammatory conditions, the dual production of NO from NO synthase and the high concentrations of luminal NO derived from dietary nitrate could be particularly potent in the progression of this disease. Therefore, therapeutic intervention aimed at reducing the production of NO may be useful in preventing the progression of BO to AC.

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Conflict of Interest Statement: None declared.

References

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