The acute phase protein haptoglobin is locally expressed in arthritic and oncological tissues

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The acute phase protein haptoglobin is locally expressed in arthritic and oncological tissues.

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Haptoglobin is an acute phase protein known to be highly expressed in the liver. Recently, we showed increased local arterial haptoglobin expression after flow-induced arterial remodelling and found that haptoglobin is involved in cell migration and arterial restructuring probably through accumulation of a temporary gelatine matrix. Since cell migration and matrix turnover are important features in the pathology of arthritis and cancer, we hypothesised that haptoglobin is also expressed in arthritic and oncological tissues. In this study, we investigated local haptoglobin expression in arthritic rats (n=12) using semi-quantitative PCR, western blotting and studied haptoglobin mRNA localisation in human kidney tumours (n=3) using in situ hybridisation. The arthritic rats demonstrated an increase of haptoglobin mRNA (2.5-fold, p<0.001) and protein (2.6-fold, p<0.001) in the arthritic achilles tendon. Haptoglobin protein was also increased in the arthritic ankle (2.6-fold, p<0.001) but not in the non-arthritic knee. In human kidney tumours, tumour and stromal cells produced haptoglobin mRNA. This study shows that the liver protein haptoglobin is, besides the artery, also expressed in arthritic and oncological tissues recognised for enhanced cell migration and matrix turnover.

Introduction

Haptoglobin is mainly produced in the liver but expression can also be induced in various other tissues. Recently, we demonstrated that the acute phase protein haptoglobin is a natural inhibitor of collagen degradation and is locally expressed in fibroblasts of the arterial wall. Haptoglobin plays an important role in cell migration and arterial restructuring. Collagen turnover is an important feature in many physiological processes like growth and wound healing. Enhanced collagen degradation is observed and often causally related with severe tissue destruction or malfunction as can be seen in the pathological processes of arthritis and cancer. The importance of haptoglobin in cell migration and extracellular matrix degradation suggests a role for haptoglobin in arthritis and cancer. Both disease processes are characterised by increased cell migration and degradation of the extracellular matrix. Invasion of arthritic fibroblast-like synoviocytes rapidly destroys a cartilage matrix. In cancer, the migration of cells plays a role in tumor angiogenesis, progression and metastasis.

Although sero-epidemiological studies reported increased haptoglobin serum levels during arthritis and carcinogenesis, local expression of haptoglobin in arthritic and oncological tissues has not been studied before. We hypothesised that local expression of haptoglobin will increase in arthritic and oncological tissues in which cell migration and matrix remodelling are important features.

In the present study, we investigated local expression of haptoglobin in arthritis and cancer and describe that haptoglobin was locally expressed in arthritic and oncological tissues in which extracellular matrix turnover and cell migration are predominant features.

Material and methods

Tissue material

Arthritic rats: In 6-8 weeks old inbred male Lewis rats (n=6), arthritis was induced by one single intradermal injection of 5 µg/ml heat-killed Mycobacterium tuberculosis (strain H37Ra, Difco) in Freund’s incomplete adjuvant (Difco) in the base of the tail; 6 additional rats were used as control. The rats were terminated after 6 weeks. The arthritic ankle and Achilles tendon as well as the unaffected knee were removed, snap-
frozen in liquid nitrogen and stored at –80°C for RNA and protein extraction.

All investigations conformed to the Guide for the Care and Use of Laboratory Animals (NIH publication No.85-23, 1985) and were approved by the ethical committee on animal experiments of the University Medical Center Utrecht.

**Human kidney tumours:** Surgical specimens from human Grawitz kidney tumours were immediately snap-frozen in liquid nitrogen and stored at –80°C for in situ hybridisation (n=3).

**RNA and protein extraction**
The frozen tissue samples were grinded in liquid nitrogen, using a pestle and mortar. RNA and protein were extracted using 1 ml Tri-pure™ Isolation Reagent (Boehringer Mannheim) according to manufacturer’s protocol.

**Semi-quantitative RT-PCR**
Specific sets of primers were constructed using software at CMBI (Nijmegen). Reverse transcription was carried out with 500 ng total RNA using superscript II (Life) according to manufacturer’s protocol. Isolation Reagent was performed according to manufacturer’s protocol.

**In situ hybridisation**
Human haptoglobin cDNA in pGEM-T Easy Vector was linearised and used as template to obtain DIGoxigenin (DIG, Roche) labelled RNA probes according to manufacturers protocol. Tissue segments were cut and used as template to obtain DIGoxigenin (DIG, Roche) labelled RNA probes according to manufacturers protocol. Human kidney tumours: Surgical specimens from human Grawitz kidney tumours were immediately snap-frozen in liquid nitrogen and stored at –80°C for in situ hybridisation (n=3).

**Results**
Local haptoglobin expression in arthritic tissue
Haptoglobin expression in the arthritic rats (n=12) was investigated using the affected ankle and achilles tendon and the unaffected knee. Haptoglobin mRNA expression was 2.5-fold increased in the achilles tendon in arthritic rats (p<0.001) (Figure 1A). Haptoglobin mRNA levels in the ankle revealed large fluctuations within the two groups but showed no significant differences between the arthritic and control rats (p=0.51) (figure 1A). No significant difference was found in haptoglobin mRNA levels in the

**Statistics**
Data are presented as mean±sem. The arthritic rats were analysed using the Mann-Whitney U test. P values <0.05 were considered as statistically significant.
Haptoglobin expression in arthritis and cancer

Recently, we have identified haptoglobin as an essential factor for cell migration. Haptoglobin knockout cells demonstrated impaired migration that could be restored by supplementation of exogenous haptoglobin to the cells. Although serum haptoglobin levels have previously been associated with the progression and outcome of arthritis and cancer, local haptoglobin synthesis has not been studied before in these pathological tissues. As cell migration is believed to be a tightly controlled process that depends on matrix degradation in the immediate surrounding of cells, we investigated in the present study the local expression of haptoglobin in arthritic and oncological tissues.

In arthritic rats, the affected ankle and Achilles tendon showed significantly increased levels of local haptoglobin expression. No differences were found in the unaffected knee demonstrating that increased haptoglobin protein levels in the affected joints are the result of local haptoglobin expression and not due to extravasation of unaffected knee between arthritic and control rats. Western blotting revealed that haptoglobin protein expression was 2.6-fold increased in both the arthritic ankle and the arthritic Achilles tendon (p<0.001) from arthritic rats (figure 1B+C). No differences in haptoglobin protein expression were found in the unaffected knee.

Haptoglobin expression in oncological tissue. In situ hybridisation on human Grawitz tumours (n=3) demonstrated haptoglobin expression by tumour cells located in invasive parts of the tumour (figure 2A+C). Alternate sections hybridised with a control sense probe showed no staining (figure 2B). Interestingly, positive staining was also found in surrounding stromal tissue (figure 2D) by macrophages and fibroblast-like cells (data not shown).

Discussion

Cell migration and collagen degradation are key events in arthritis, tumour progression and tumour metastasis. Enhanced degradation of collagen is required for cell migration to occur. Recently, we have identified haptoglobin as an essential factor for cell migration. Haptoglobin knockout cells demonstrated impaired migration that could be restored by supplementation of exogenous haptoglobin to the cells. Although serum haptoglobin levels have previously been associated with the progression and outcome of arthritis and cancer, local haptoglobin synthesis has not been studied before in these pathological tissues. As cell migration is believed to be a tightly controlled process that depends on matrix degradation in the immediate surrounding of cells, we investigated in the present study the local expression of haptoglobin in arthritic and oncological tissues. Previous studies have demonstrated increased haptoglobin levels in the serum during arthritis.

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haptoglobin from the serum. Fibroblast-like synoviocytes of human arthritic tissue migrate in vivo into cartilage matrix resulting in a destructive pannus as is found in human arthritic tissue. These fibroblast-like synoviocytes are the main producers of the high local levels of MMPs that correlate with the severity of the lesion and are mainly produced in arthritic tissue. We infer that local haptoglobin expression facilitates cell migration in the cartilage and may therefore play a role in the progression of arthritis.

Haptoglobin mRNA was also locally expressed in kidney tumour tissue. Cells staining positive for haptoglobin mRNA were mainly found in the invasive part of the tumour and in the surrounding stroma. Haptoglobin protein levels were not measured in the tumour samples due to contamination with blood residues. The localisation of haptoglobin correlated with the earlier described expression pattern of MMP-2, which is mainly synthesised by fibroblasts in the stroma surrounding the tumours although the active protein can also be found around tumour cells. Furthermore, MMP synthesis by surrounding stromal cells is necessary for tumour metastasis indicating the important role of stromal-derived MMP activity. Increased expression of MMPs has been associated with tumour invasion, metastasis and angiogenesis and the controlled degradation of surrounding stromal matrix appears to be essential for these three processes.

Increased local haptoglobin expression in pathological processes and even appear to have prognostic values for some diseases. Increased local haptoglobin expression with other glycosylation patterns might therefore reflect an altered function compared to haptoglobin produced in the liver.

A limitation of this study is that it is purely descriptive and we can only speculate about the exact function of haptoglobin in these tissues. However, haptoglobin is locally produced in both pathological tissues and is described to be involved in cell migration and matrix degradation.

In summary, we have investigated local haptoglobin expression in arthritis and cancer where cell migration and matrix remodelling are important features in arthritis and cancer, this suggests a local role for haptoglobin in these processes and supports a role for haptoglobin in the initial response to tissue injury. This is in accordance with studies in haptoglobin knockout mice that showed a delayed response to arterial or kidney injury.

In summary, we have investigated local haptoglobin expression in arthritis and cancer where cell migration and matrix remodelling are important features and describe an upregulation of local haptoglobin expression in arthritic and oncological tissues.

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References


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