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Haemorrhoids and transient receptor potential vanilloid 1

Haemorrhoids are extremely common, and approximately 10 million people are affected by haemorrhoidal disease in the USA.¹ Many causes have been proposed to explain the pathogenesis of symptomatic haemorrhoids. The pathophysiological theory, made popular by Thomson's studies, in which the elastic support of the anal cushions is thought to be broken, is currently the best accepted.¹

The function and control of anorectal arteriovenous anastomoses remain unclear as does the existence of individual susceptibility to haemorrhoidal disease, which is influenced by socioeconomic, cultural, and psychological factors.¹ Previous studies have established that intake of hot pepper and alcohol abuse may influence disease progression and cause acute exacerbation of haemorrhoidal disease.²

We investigated haemorrhoidal disease and transient receptor potential vanilloid 1 (TPVR1), the receptor for capsaicin, the spicy component contained in plants of the genus *Capsicum*.³ TPVR1 is a non-selective cation channel that is expressed and stimulates

Figure 1 Immunohistochemistry of transient receptor potential vanilloid 1 (TPVR1) protein expression in tissue sections from the normal anal region (A, D) and in haemorrhoid samples (B, C, E, F, G). In the normal anal region, weak TPVR1 (A, arrow) immunoreactivity was present in a few epithelial cells and some endothelial cells of small and medium sized arteries (D). In contrast, haemorrhoid tissue sections exhibited intense TPVR1 immunoreactivity, especially in the group of patients with acute presentation of the disease (B, E, F) (group 1). Intense staining signals were primarily localised in the stratified squamous epithelium of the anal mucosa near the anorectal junction (B). Columnar epithelium of the rectum showed very weak or no TPVR1 immunoreactivity (B, insert). TPVR1 was also present in the endothelial wall of the small vessels (arteries and veins) and in the nerve fibres of the anal canal (E, F). Similar patterns of distribution but with low intensity were detectable in group 2 (C, G). Arrows indicate positive signals. Original magnification: $\times 20$ (A–D, G); $\times 40$ (E, F).

Figure 2 Statistical analysis demonstrated a significant difference between group 1 and group 2 in per cent transient receptor potential vanilloid 1 (VR1) immunoreactivity (group 1, mean (SD) 10.4 (6.5); group 2, mean (SD) 50.8 (35.4); $p < 0.05$). In the normal anal region samples, signals were too weak for measurement. Group 1, haemorrhoids with histological evidence of thrombosis; group 2, haemorrhoids without histological evidence of thrombosis.

primary sensory neurones. Noxious heat and capsaicin directly activate TPVR1. TPVR1 acts as an ion channel with increased permeability to Na^+ and Ca^{2+} ions, which in turn causes neuronal depolarisation with sensation of burning pain and release of neuropeptides, such as substance P and calcitonin gene related peptide, thereby contributing to "neurogenic inflammation".^{4,5}

For this study, tissue samples were obtained from patients undergoing haemorrhoidectomy (Milligan-Morgan operation) at the University Hospital of Heidelberg (Heidelberg, Germany) for haemorrhoidal disease (groups 1 and 2) or from patients undergoing Miles operation for colorectal cancer or autopsy but without clinical signs of haemorrhoidal disease (control group; group 3). After histological examination, patients with haemorrhoidal disease were subdivided into two groups: haemorrhoidal disease with histological signs of thrombosis (group 1, $n = 10$; one female, nine males; median age 55 years (range 39–82)) and without histological signs of haemorrhoidal thrombosis (group 2, $n = 5$; one female, four males; median age 64 years (range 53–79)). In addition, as a control (group 3), six patients (three females, three males; median age 52 years (range 18–60)) with normal haemorrhoidal tissue were included.

Paraffin embedded tissue sections were subjected to immunostaining using the Dako Envision+ system (Dako Diagnostics AG, Zurich, Switzerland) using polyclonal anti-TPVR1 antibodies (Chemicon International, Inc, Temecula, California, USA) (dilution 1:1000).⁶ A computerised image analysis system with a standardised setting was used to analyse immunolabelled tissue sections using Image Pro-Plus 4.1 software (Media Cybernetics, Maryland, USA).⁷ The Mann-Whitney two sample test was used for statistical analysis (Prism 3.0; GraphPad Software, Inc, San Diego, California, USA). Figure 1 demonstrates the pattern of distribution of TPVR1, with highly significant levels of TPVR1 immunoreactivity detected in tissue samples of patients in group 1 (fig 2). This study demonstrates that TPVR1 activation is involved in the course of the disease.

If we assume that signs of thrombosis are an expression of disease severity, as we have learned from clinical experience, high TPVR1

expression detected in patients operated on for acute haemorrhoidal disease and with histological signs of thrombosis suggests the involvement of this receptor in the pathogenesis of haemorrhoids. In addition, recent studies suggest that anorectal vascular permeability may play a role in acute exacerbation of haemorrhoids.⁸ Interestingly, TPVR1 can influence the permeability and tone of the vessels by interacting with the calcium channel of the cells in different tissues.^{9,10}

In light of the present results it is possible to postulate that activation of the vanilloid receptor in haemorrhoid tissue might be one of the potential mechanisms that generate "acute" presentation of the disease. In addition, the presence of capsaicin may generate activation of capsaicin sensitive primary afferent neurones with neuropeptide release. However, the question remains why some individuals have different sensitivities and consequently different levels of TPVR1 expression. High levels of TPVR1 in the anorectal region might be the principal disorder that contributes to the pathogenesis of the disease. On the other hand, the presence of TPVR1 may only be a "symptom" or consequence of the disease. Further studies are needed to clarify this issue.

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Anti-TNF- α for treatment of amyloidosis associated with Crohn's disease

We wish to comment on the case reported by Iizuka and colleagues (*Gut* 2006;**55**:744–5) of a patient who experienced a reduction in creatine levels from 4 to 2 mg/dl after three weeks of treatment with infliximab for amyloidosis associated with Crohn's disease. Unfortunately, no details were provided of other concomitant factors, such as volume depletion or drugs, which may have affected baseline creatinine levels and their later course, irrespective of amyloidosis, or of the results of proteinuria observed in 1992.

In our experience,¹ anti-TNF- α therapy has a rapid effect on proteinuria but not on renal function. In our patients it took several months (the most rapid decrease was from 1.6 to 0.94 mg/dl in three months) for serum creatinine levels to decrease by (mean (SD) percentage since baseline) 8.5 (16.6)% (range –21.4 to 39.4) after 70 (44) weeks (range 2–120), and only 36% of patients experienced a 20% reduction or greater.

We should also like to comment on the choice of anti-TNF for the management of amyloidosis in patients with quiescent Crohn's disease but active spondyloarthropathy.

One of our patients was diagnosed by clinical, endoscopic, and histological criteria with spondyloarthropathy associated with Crohn's disease in 1988 at the age of 20 years. His intestinal disease was moderately active at the beginning whereas his spondyloarthropathy remained highly active, causing severe restriction in the whole spine, shoulders, and hips. He underwent arthroplasty of both hips in 1995 and 1997. In 1998, when his intestinal disease had been inactive for more than five years, he developed nephrotic syndrome (proteinuria of 11.72 g/day and normal renal function). Rectal biopsy was negative but kidney biopsy showed extensive glomerular and arteriolar AA amyloid deposits. At the beginning of 2001 his renal function started to decline and treatment was initiated with etanercept 25 mg, twice weekly, with excellent clinical and analytical results (fig 1). Twelve months later repeated episodes of intestinal activity commenced, with later worsening of the proteinuria and renal function. After 29 months, etanercept was replaced by infliximab (5 mg/kg) but his status continued to worsen despite haemodialysis and ileal resection, with several interruptions of infliximab until his death 14 months later with severe pancytopenia due to progression of amyloidosis, as shown at autopsy.