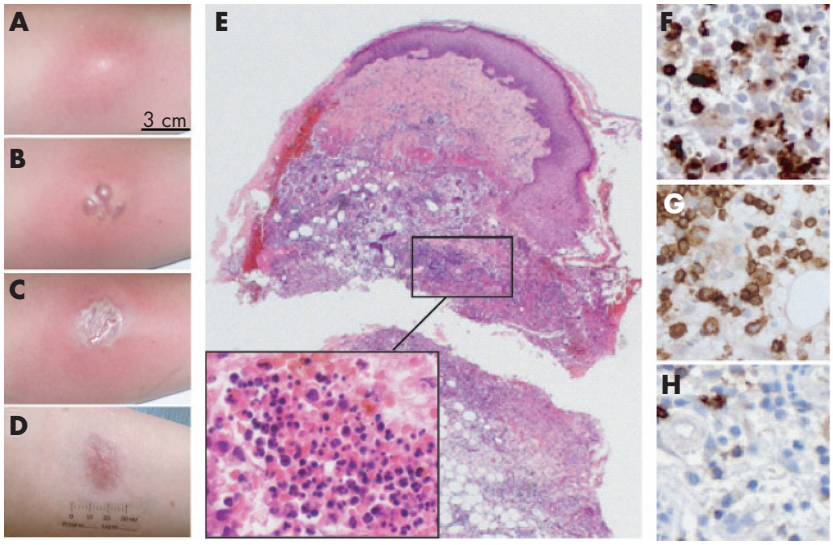

Figure 1 Macroscopic and microscopic appearances of the skin lesions. (A) A prolonged erythematous reaction developed at each injection site in this patient. These subsequently (B) blistered and (C) ulcerated. (D) Ulcers completely re-epithelialised after sequential treatment with infliximab, clobetasol, and topical tacrolimus. (E) Biopsies taken from the margin of the lesion showed granulation tissue, active inflammation in the subcutis, fibrin exudation into the superficial dermis, and pseudoepithelialomatous hyperplasia. High power images (inset) revealed a predominantly neutrophilic leucocyte infiltrate. Immunostaining of the biopsy sample for (F) the neutrophil marker myeloperoxidase, and T lymphocyte markers (G) CD4 and (H) CD25.

formation by day 6 (fig 1B). The patient was otherwise apyrexial and systemically well, with no leucocytosis or gastrointestinal symptoms.

The blisters, 3 cm in diameter, were drained (fig 1C). Aspirated fluid was sterile and contained micromolar concentrations of interleukin 8, levels 1000-fold higher that those reported in skin window[1] or blister[2] models of acute inflammation. Other cytokines were only minimally elevated; anti-inflammatory interleukin 10 and transforming growth factor β were barely detectable.[3] By day 8, two 30×25 mm ulcers with tender, indurated, purple borders had developed at each injection site, appearances highly suggestive of pyoderma gangrenosum. Histological analysis of the ulcer margin revealed dermal abscesses with a neutrophil rich acute inflammatory infiltrate extending into the subcutis (fig 1E), marked oedema, and fibrin exudation. Immunohistochemical analysis confirmed a predominance of neutrophils (fig 1F) alongside $CD4^+$ (fig 1G) and $CD25^-$ (fig 1H) T lymphocytes, with minimal changes in Langerhans cell, monocyte, and macrophage populations. A 5 mg/kg infusion of infliximab was commenced, which markedly reduced inflammation within 12 hours. The ulcers responded partially to four weeks of clobetasol propionate 0.05% but completely re-epithelialised after two weeks of treatment with topical tacrolimus 0.03% (fig 1D). The patient is currently well.

We subsequently discovered that six years previously this patient had developed a similar extreme reaction to infection of a surgical wound on her foot. This was treated with antibiotics and steroids but eventually required debridement. Conversely, she did not exhibit a generally abnormal response to trauma, as multiple venepunctures and previous tattooing, piercings, and acupuncture healed normally, arguing against classical pathergy.

The abnormally protracted inflammatory response was seen in two patients with ulcerative colitis but not in 13 healthy subjects or 12 patients with Crohn's disease,[1] implicating a hyperinflammatory reaction to *E coli* in this condition. This might represent an underlying pathological mechanism. The extraordinary severity in this patient could be idiosyncratic or a more general phenomenon in ulcerative colitis. The previous grossly abnormal response to sepsis in her foot favours the former.

Our interpretation of the pathogenesis of these local lesions is that failure to terminate proinflammatory interleukin 8 production led to sustained neutrophil accumulation. Absence of regulatory T cells and minimal anti-inflammatory cytokine concentrations[3] suggests deficient activation of immunoregulatory mechanisms.

Pyoderma gangrenosum is an ulcerating neutrophilic dermatosis of unknown aetiology. It develops in 5% of inflammatory bowel disease patients and was once considered pathognomonic of ulcerative colitis.[4] It is not thought to be infective in origin, and in a minority of cases may be a pathergic reaction to trauma.[5] The lesions in this patient resembled pyoderma gangrenosum, suggesting that bacterial products might predispose to its development.

This case is instructive as lesions were induced by *E coli* in a patient with a chronic inflammatory disease of the large bowel where such organisms are copious. The pathological response to bacteria points to possible mechanisms underlying the inflammatory processes causing ulcerative colitis, as well as its cutaneous manifestation of pyoderma gangrenosum.

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Supplementary fig 1 can be viewed on the *Gut* website at http://www.gutjnl.com/supplemental

References

1 **Marks DJ**, Harbord MW, MacAllister R, *et al*. Defective acute inflammation in Crohn's disease: a clinical investigation. *Lancet* 2006;**367**:668–78.
2 **Day RM**, Harbord M, Forbes, et al. Cantharidin blisters: a technique for investigating leukocyte trafficking and cytokine production at sites of inflammation in humans. *J Immunol Methods* 2001;**257**:213–20.
3 **Yagnik DR**, Evans BJ, Florey O, *et al*. Macrophage release of transforming growth factor beta1 during resolution of monosodium urate monohydrate crystal-induced inflammation. *Arthritis Rheum* 2004;**50**:2273–80.
4 **Crowson AN**, Mihm MC Jr, Magro C. Pyoderma gangrenosum: a review. *J Cutan Pathol* 2003;**30**:97–107.
5 **Dwarakanath AD**, Yu LG, Brookes C, *et al*. 'Sticky' neutrophils, pathergic arthritis, and response to heparin in pyoderma gangrenosum complicating ulcerative colitis. *Gut* 1995;**37**:585–8.

A pilot study in patients with established advanced liver fibrosis using pirfenidone

Cirrhosis represents the third cause of mortality among Mexican people of productive age.[1] Several drugs have been tested in the clinical scenario[2 3] although conclusive evidence concerning drug efficacy has proven elusive.

PFD is an orally bioavailable pyridone derivative (5-methyl-1-phenyl-2-(1H)-pyridone) that affects a variety of profibrogenic cytokines and its mechanism of action mostly resides in its anti-inflammatory and anti-fibrotic activity.[4–6] Here we present data obtained from a pilot clinical trial evaluating the safety and efficacy of PFD in 15 patients with established advanced liver disease caused by hepatitis C virus chronic infection.

Table 1 Treatment outcomes determined by fibrosis stage

Patient No	Genotype	Ishak staging score	
		Baseline	12 months
1	1b	4	1
2	1b	2	1
3	1b	6	6
4	ND	4	2
5	1b	4	4
6	1b	4	1
7	3a	6	6
8	1b	6	2
9	1a–1b	1	1
10	1b	6	6
11	1b	2	2
12	1	5	5
13	2a–2c	4	4
14	2a–2c	6	6
15	1a	5	5

HCV genotyping was conducted by specific viral DNA sequencing. Fibrosis stage was estimated using the Ishak-Kamal index at baseline (0) and 12 months after PFD treatment.
ND, not determined.

This is the first report showing improvements in liver histology (that is, necrosis, inflammation, steatosis, fibrosis, and cell regeneration) 12 months after PFD therapy. Colour Doppler ultrasound guided liver biopsies were obtained at baseline and after 12 months of PFD treatment and evaluated for stage of fibrosis and grade of activity according to the modified histological activity index (HAI) of Knodell and Ishak fibrosis stage. Two pathologists who were blinded to the sequence and clinical and biochemical characteristics of the patients evaluated the biopsies.

Fifteen patients who gave written informed consent and had no history of alcohol intake were included in the final analysis based on the size of the liver biopsy satisfying international criteria. None of these patients had taken antiviral therapy previously. Mean age was 57 years (range 48–70) and there were five males. PFD was well tolerated at the dose used in this study (1200 mg/day), and only 15% of patients developed photosensitivity, rash and itching, and gastrointestinal symptoms such as nausea, abdominal discomfort, and diarrhoea. After 2–3 months of PFD therapy, adverse reactions disappeared.

Histological differences were noted in the liver biopsies at the end of therapy. In 53.3% of patients a 2 point or greater reduction in the HAI necroinflammatory score was noted. Steatosis decreased in 60% of patients, remained unchanged in 26.7%, and worsened in 13.3%. Liver cell regeneration was detected in 70% of patients with different degrees of anti-proliferating cell nuclear antigen immunostaining. Fibrosis was reduced in 30% of patients by the end of 12 months of treatment (table 1). Representative photomicrographs of liver biopsies from two different patients are shown in fig 1 where steatosis and chronic hepatitis with portal tract inflammation, piecemeal necrosis, and necroinflammatory foci per lobule were clearly lessened after 12 months of treatment.

HCV RNA levels were measured at six months; nine patients had a decrease in viral load, two patients remained unchanged, and four patients displayed an increase in viral load compared with baseline. No patient had a sustained virological response. Median (range) values for changes in alanine aminotransferase (ALT) levels over time are given in fig 1E. A tendency to normal values was evident; 4/15 (27%) HCV patients had normalisation of ALT, 7/15 (47%) had decreased ALT values, one showed no change (7%), and three patients showed a modest increase in ALT (20%). Ultrasonographic measurements by colour Doppler imaging indicated no significant differences between spleen size before and after PFD treatment. None the less, hepatic echogenicity decreased significantly. There was no significant difference in portal vein diameter after PFD treatment but a noticeable increase in portal vein flow velocity was observed 12 months after PFD ($p<0.05$).

An SF-36 survey, self-administered by patients, demonstrated an improvement in quality of life.[7]

Real Time PCR was used to detect gene expression of key molecules involved in collagen turnover. mRNAs coding for profibrogenic molecules such as Col α1, transforming growth factor β, and tissue inhibitor of metalloproteinase 1 were markedly downregulated at the end of treatment.

Although promising, these results need to be verified and extended, in the context of a placebo controlled, double blind clinical trial.

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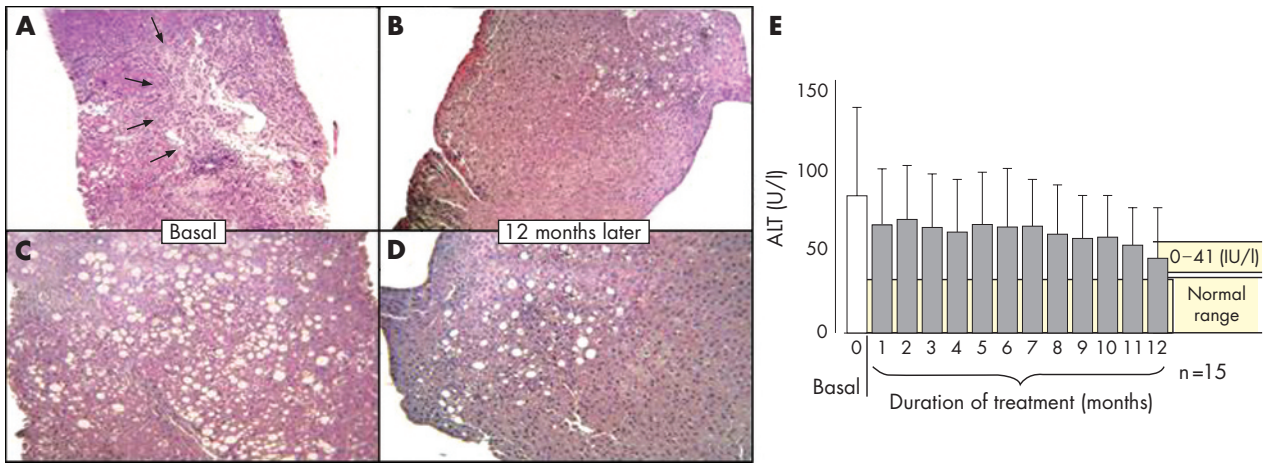

Figure 1 Liver tissues (5 μm) were stained with haematoxylin-eosin. (A) Patient 02/011 (hepatitis C virus (HCV) genotype 1b). Chronic hepatitis with portal tract inflammation, piecemeal necrosis, and necroinflammatory foci per lobule are clearly seen before PFD treatment. (B) Same patient as in (A), 12 months after PFD treatment. Decreased necroinflammatory activity is noticeable. (C) Patient 02/008 (HCV genotype 3a) shows marked steatosis in the first biopsy. (D) Same patient as in (B), 12 months later. Liver steatosis has decreased markedly. (E) Gradual decrease in alanine aminotransferase (ALT) levels during the course of PFD treatment (mean (SD) of 15 patients).

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References

1 http://www.inegi.gob.mx/prod_serv/contenidos/espanol/bvinegi/productos/continu1, as/vitales/demograficas/2003/cuademo16.pdf (last accessed 17 August 17 2005).
2 **Friedman SL**, Maher JJ, Bissell DM. Mechanisms and therapy of hepatic fibrosis: report of the AASLD Single Topic Basic Research Conference. *Hepatology* 2000;**32**:1403–8.
3 **Pinzani M**, Rombouts K, Colagrande S. Fibrosis in chronic liver diseases: diagnosis and management. *J Hepatol* 2005;**42**:S22–36.
4 **Garcia L**, Hernandez I, Sandoval A, *et al*. Pirfenidone effectively reverses experimental liver fibrosis. *J Hepatol* 2002;**37**:797–805.
5 **Di Sario A**, Bendia E, Macarri G, *et al*. The anti-fibrotic effect of pirfenidone in rat liver fibrosis is mediated by downregulation of procollagen alpha1(I), TIMP-1 and MMP-2. *Dig Liver Dis* 2004;**36**:744–51.
6 **Armendariz-Borunda J**, Islas-Carbajal MC, Meza E, *et al*. A pilot study of a novel anti-inflammatory and anti-fibrotic agent, pirfenidone, in patients with liver cirrosis. *Hepatology* 2003;**38**(suppl 1):308A.
7 **Bonkovsky HL**, Woolley JM. Reduction of health-related quality of life in chronic hepatitis C and improvement with interferon therapy. The Consensus Interferon Study Group. *Hepatology* 1999;**29**:264–70.

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

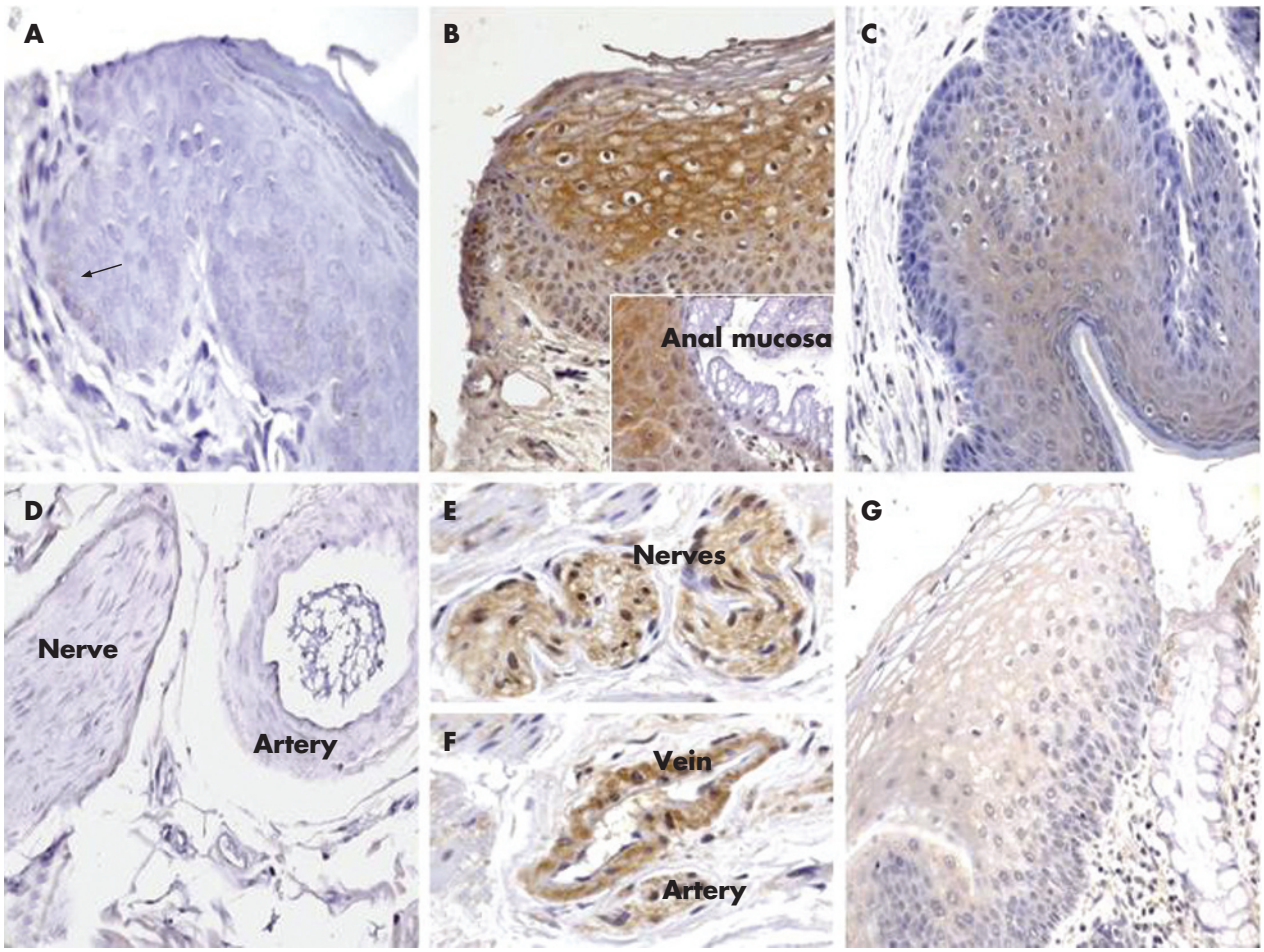

Figure 1 Immunohistochemistry of transient receptor potential vanilloid 1 (TPVR1) protein expression in tissue sections from the normal anal region (A, D) and in haemorrhoid samples (B, C, E, F, G). In the normal anal region, weak TPVR1 (A, arrow) immunoreactivity was present in a few epithelial cells 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: ×20 (A–D, G); ×40 (E, F).