PANCREATIC CANCER

Vanilloids in pancreatic cancer: potential for chemotherapy and pain management

M Hartel, F F di Mola, F Selvaggi, G Mascetta, M N Wente, K Felix, N A Giese, U Hinz, P Di Sebastiano, M W Büchler, H Friess

Gut 2006;**55**:519–528. doi: 10.1136/gut.2005.073205

See end of article for authors' affiliations

Correspondence to: Dr H Friess, Department of General Surgery, Im Neuenheimer Feld 110, D-69120 Heidelberg, Germany; helmut.friess@med.uni-heidelberg.de

Revised version received 23 August 2005
Accepted for publication 6 September 2005

Background: Success of chemotherapy and alleviation of pain are frequently less than optimal in pancreatic cancer patients, leading to increasing interest in new pharmacological substances, such as vanilloids. Our study addressed the question of whether vanilloids influence pancreatic cancer cell growth, and if vanilloids could be used for pain treatment via the vanilloid 1 receptor (VR1) in pancreatic cancer patients.

Methods: In vitro, the effect of resiniferatoxin (vanilloid analogue) on apoptosis and cell growth in pancreatic cancer cells—either alone, combined with 5-fluorouracil (5-FU), or combined with gemcitabine—was determined by annexin V staining, FACS analysis, and MTT assay, respectively. VR1 expression was evaluated on RNA and protein level by quantitative polymerase chain reaction and immunohistochemistry in human pancreatic cancer and chronic pancreatitis. Patient characteristics—especially pain levels—were registered in a prospective database and correlated with VR1 expression.

Results: Resiniferatoxin induced apoptosis by targeting mitochondrial respiration and decreased cell growth in pancreatic cancer cells without showing synergistic effects with 5-FU or gemcitabine. Expression of VR1 was significantly upregulated in human pancreatic cancer and chronic pancreatitis. VR1 expression was related to the intensity of pain reported by cancer patients but not to the intensity of pain reported by patients with chronic pancreatitis.

Conclusions: Resiniferatoxin induced apoptosis in pancreatic cancer cells indicates that vanilloids may be useful in the treatment of human pancreatic cancer. Furthermore, vanilloid might be a novel and effective treatment option for neurogenic pain in patients with pancreatic cancer.

Pancreatic cancer is the fourth or fifth leading cause of cancer related death in most Western industrialised countries, with an average survival after diagnosis of 3–6 months.[1] Efforts have been directed towards the development of adjuvant and neoadjuvant therapies in an attempt to improve outcome.[2–6] Unfortunately, the toxicity of chemotherapeutic agents such as 5-fluorouracil (5-FU) and gemcitabine, and inherent resistance to these agents in pancreatic cancer, are still reasons for unsatisfactory survival rates. Therefore, novel therapeutic strategies are of major interest in the treatment of patients with pancreatic cancer.

As ductal adenocarcinoma of the pancreas is one of the most resistant forms of cancer, apoptosis is a desirable end point for both chemoprevention and chemotherapy.[7 8] Vanilloids such as capsaicin and resiniferatoxin (RTX) have been demonstrated to induce apoptosis in different transformed cell types in vitro and in animal models of carcinogenesis.[9–16] Capsaicin was shown to protect against experimentally induced mutagenesis and tumorigenesis in various model systems, including benzo(a)pyrene induced pulmonary adenomas and carcinomas in mice, 4-nitroquinoline 1-oxide induced tongue carcinoma in rats, and vinyl carbamate induced skin tumours in mice.[15 17–19] Topical application of capsaicin suppressed phorbol ester induced activation of nuclear factor κB/Rel and activator protein 1 transcription factors in mouse epidermis.[12 20] Suppression of these transcriptional factors by capsaicin may account for its chemopreventive effects on both mouse skin tumorigenesis and inflammation.

There are several potential cellular targets for vanilloids in various cell types, many of which can trigger acute cytotoxic effects capable of inducing apoptosis independently from the vanilloid receptor. In addition to their inhibitory effects on plasma membrane NADH oxidoreductase activity causing apoptosis, vanilloids can block electron transfer at the NADH:coenzyme Q oxidoreductase of the mitochondrial electron transport chain, presumably by acting as a coenzyme Q antagonist.[16 21–24] Capsaicin and RTX are structurally similar to the cyclic portion of coenzyme Q. Various studies have shown that mitochondria and mitochondrial derived factors are involved in the process of apoptosis.[16 25–27] Therefore, mitochondria are targets for cancer prevention and chemotherapy because chemotherapeutic cancer agents may modulate or interfere with mitochondrial functions to promote mitochondrial membrane permeability and cell death.[11] Vanilloids alter membrane structure and function, promote the generation of reactive oxygen species, depolarise the mitochondria of intact cells, and induce apoptosis in transformed cells and in activated T cells.[7 8 15 18 19 28–32] Vanilloid induced apoptosis in human cutaneous squamous cell carcinoma appears to involve inhibition of mitochondrial respiration.[9] These observations suggest that vanilloids should be considered as potential anticancer agents.

In addition to possessing the properties described above, vanilloids are potent neurotoxins via the vanilloid 1 receptor (VR1), promoting apoptosis and necrosis in primary sensory neurones when injected subcutaneously.[33–38] As control of cancer pain with currently available analgesics is frequently less than optimal, vanilloids may provide a therapeutic

Abbreviations: VR1, vanilloid 1 receptor; FACS, fluorescence activated cell sorter; 5-FU, 5-fluorouracil; VAS, visual analogue scale; HRP, horseradish peroxidase; PCR, polymerase chain reaction; DCFH-DA, 2′,7′-dichlorodihydroflourescein diacetate; DCF, 2′,7′-dichloro-fluorescein

benefit in the management of certain types of neurogenic pain and inflammation.[26 35 39–42]

Until now, the influence of vanilloids, such as RTX, and the role of the vanilloid receptor in ductal pancreatic adenocarcinoma have not been evaluated. We tested whether treatment with RTX induces apoptosis and decreases growth in pancreatic cancer cell lines, and whether RTX may possess synergistic effects when used with gemcitabine or 5-FU for chemotherapy. Additionally, we examined expression of the vanilloid receptor in human pancreatic cancer and in the normal pancreas, especially with regard to correlation with clinical characteristics such as pain in cancer patients. Although some studies have shown that VR1 may not be involved in the apoptotic effect of vanilloids in cancer cells, its expression may have an impact in nerves, and therefore it may also be useful in pain therapy with vanilloids for patients with pancreatic cancer.

MATERIALS AND METHODS

Patients and tissue collection

Normal human pancreatic tissue samples were obtained through an organ donor programme from 19 individuals who were free of any pancreatic disease (table 1). Mean age of the organ donors was 54.1 years (SD 12.5) (table 2). Pancreatic cancer tissue samples were obtained from 32 patients (four females, 28 males) undergoing a Whipple resection for ductal adenocarcinoma of the pancreas. Mean age of the patients with pancreatic cancer was 64.1 years (SD 12.1). According to the international classification of the UICC, there were six pancreatic cancers with stage I, six with stage II, 16 with stage III, and four with stage IV. Tumour grading was well differentiated in seven cases, moderately differentiated in 15 cases, and undifferentiated in 10 cases. Chronic pain was assessed in all patients before surgery using a standardised questionnaire. The degree of chronic pain was defined as follows: 0, no pain; I, mild pain (abdominal discomfort or pain not requiring analgesics or not disabling, 1–3 on a visual analogue scale (VAS)); II, moderate pain (pain controlled by non-narcotic analgesics, VAS 4–6); and III, severe pain (pain that required narcotic analgesics and was disabling, VAS 7–10). Median survival rate of all operated pancreatic cancer patients was 15.6 months (SD 8.7) (tables 1, 2).

Chronic pancreatitis tissue samples were obtained from 19 patients (seven females, 12 males) undergoing a duodenal preserving resection of the pancreas head. Mean age of the patients with chronic pancreatitis was 58.3 years (SD 8.9) (tables 1, 2).

Freshly removed tissue samples were cut in the operating room on surgical removal and randomly divided for histological analysis (immediately fixed in paraformaldehyde solution for 12–24 hours and paraffin embedded for immunohistochemistry) or were snap frozen in liquid nitrogen and maintained at −80°C until further analysis. Studies were approved by the human ethic committees of the universities of Bern and Heidelberg.

Immunohistochemistry

Paraffin embedded tissue sections (4 μm in thickness) were subjected to immunostaining using the Dako Envision$^+$ System (Dako Diagnostics AG, Zürich, Switzerland). Tissue sections for each tissue sample were deparaffinised with xylene and rehydrated through graded alcohol into distilled water. Endogenous peroxidase activity was quenched by incubating the slides in 0.03% hydrogen peroxide and sodium azide, followed by washing in Tris buffered saline. Sections were then incubated overnight at 4°C with affinity purified polyclonal anti-VR1 antibody (1:1000) (Chemicon, Temecula, USA) diluted in 50 mM Tris-HCl buffer containing 1% bovine serum albumin (Carl-Roth-GmbH, Karlsruhe, Germany). These antibodies were raised in rabbits against residues 81–94 of VR1, which is a non-conserved domain among other VR1 related molecules. Bound antibody was detected with a streptavidin-biotin-horseradish peroxidase (HRP) system (Dako, Hamburg, Germany) in which slides were successively incubated with biotinylated antirabbit IgG (Dako), streptavidin-HRP, and 3-3′ diaminobenzidine. To ensure antibody specificity, control slides were incubated either in the absence of primary antibody or with a non-specific IgG antibody. In neither case was immunostaining detected. All slides were analysed by two independent observers blinded to the patient status; any differences were resolved by joint review and consultation with a third observer.

Human pancreatic cancer cell lines

Pancreatic cancer cell lines MIA PaCa-2 and Capan-1 were cultivated in complete RPMI-1640 medium containing 2 mM glutamine, 10 mM HEPES, 1 mM sodium pyruvate, penicillin/streptomycin 50 U/ml (Invitrogen, Karlsruhe, Germany), and 10% fetal bovine serum (PAN Biotech GmbH, Aidenbach, Germany) at 37°C and 7% CO_2.

The two pancreatic cancer cell lines, treated for 24 hours with 5-FU, gemcitabine, or resiniferatoxin alone (RTX; Sigma Chemical Co., St Louis, MO, USA), or with a combination of one of the chemotherapeutic drugs and RTX, were compared with each other and with the untreated control group. The chemotherapeutic compounds were used at previously established IC_{50} concentration (data not shown). To determine the concentration of RTX sufficient to promote apoptosis in approximately 50–60% of pancreatic cancer cells, cells were treated for 24 hours with RTX at graded concentrations (2, 10, and 100 μM).

Tumour cell growth analysis (MTT)

Growth of untreated pancreatic cancer cells (control) and drug treated cell cultures was measured by the MTT assay, as described previously.[43] To test for synergism between RTX and the chemotherapeutic drugs, 5-FU (IC_{50} 4.63 μM in MIA PaCa-2 and 0.22 μM in Capan-1) and gemcitabine (IC_{50} 35.87 nM in MIA PaCa-2 and IC_{50} 11.51 nM in Capan-1; Lilly Deutschland GmbH, Bad Homburg, Germany) were added to the pancreatic cancer cells, either individually or in combination with RTX (10 μM). Additionally, we performed the same experiments with reduced concentrations of all drugs (one fifth of the IC_{50} for the chemotherapeutic drugs with or without 10 μM or 2 μM RTX).

Briefly, cells were seeded at a density of 5000 cells/well in 96 well plates, grown overnight, and exposed to 5-FU or gemcitabine alone or in combination with RTX. After 24 hours of drug exposure, 3-(4, 5-methylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (Sigma-Aldrich Chemie, Deisenhofen, Germany) was added (50 μg/well) for four hours. Formazan products were solubilised with acidic isopropanol, and optical density was measured at 570 nm. Values for each experiment were converted to percentages related to the corresponding control group (100%).

Real time quantitative polymerase chain reaction (PCR)

All reagents and equipment for mRNA/cDNA preparation were purchased from Roche Applied Science (Mannheim, Germany). mRNA was prepared by automated isolation using MagNA Pure LC instrument and isolation kits I (for cells) and II (for tissues). cDNA was prepared using the First Strand cDNA Synthesis Kit for reverse transcription-PCR according to the manufacturer's instructions. Real time PCR was performed with the LightCycler FastStart DNA SYBR Green kit, as described previously.[44 45] The number of specific transcripts (VR1) was normalised to housekeeping genes

Table 1 Patient characteristics

Patient No	Sex	Age (y)	UICC stage	Survival (months)	Pain score	VR1 (mRNA copies)
Normal pancreas						
1	Female	45				10
2	Male	50				7
3	Male	49				13
4	Male	64				4
5	Female	69				2
6	Male	66				21
7	Male	61				12
8	Male	26				7
9	Male	42				15
10	Male	41				2
11	Male	35				1
12	Male	67				1
13	Female	70				2
14	Female	49				2
15	Female	57				4
16	Female	60				1
17	Female	63				13
18	Male	45				2
19	Male	69				3
Chronic pancreatitis						
1	Female	44			3	18
2	Female	36			2	7
3	Female	53			1	10
4	Male	60			3	9
5	Male	58			1	6
6	Female	57			1	33
7	Male	54			2	13
8	Male	58			1	10
9	Male	58			2	11
10	Male	58			1	12
11	Male	60			1	3
12	Male	69			2	5
13	Male	49			3	2
14	Male	63			2	62
15	Male	60			3	10
16	Male	74			2	27
17	Female	70			3	16
18	Female	65			2	6
19	Female	63			2	10
Pancreatic cancer						
1	Male	71	3	11	1	8
2	Male	54	3	15	2	16
3	Female	49	3	9	2	22
4	Male	77	3	6	2	26
5	Male	76	3	5	3	38
6	Male	73	3	8	0	6
7	Male	75	2	7	2	12
8	Male	72	1	Alive	0	5
9	Male	69	1	18	3	60
10	Male	61	3	14	3	76
11	Female	68	4	11	1	4
12	Female	71	1	27	2	18
13	Female	55	1	10	2	14
14	Male	61	2	30	2	23
15	Male	79	2	15	3	61
16	Male	69	4	9	3	42
17	Male	58	3	17	3	73
18	Male	69	2	Alive	0	0
19	Male	66	3	21	2	15
20	Male	73	3	Alive	1	1
21	Male	40	1	18	2	16
22	Male	76	3	20	0	3
23	Male	57	4	3	3	163
24	Male	23	3	19	1	24
25	Male	77	1	4	0	3
26	Male	62	4	9	1	6
27	Male	61	2	21	2	13
28	Male	54	3	26	3	79
29	Male	73	3	15	2	21
30	Male	70	3	9	0	3
31	Male	58	3	28	1	8
32	Male	55	2	25	3	100

Table 2 Relationship between characteristics of patients with pancreatic cancer and vanilloid 1 receptor (VR1) expression (mRNA copies)

Characteristic	Normal pancreas	Chronic pancreatitis	Pancreatic cancer	p Value*
Patients (n)	19	19	32	
Age (y) (mean (SD))	54.1 (12.5)	58.3 (8.9)	64.1 (12.1)	0.0527†
Sex (n)				–
Male	12	12	28	
Female	7	7	4	
Grading				0.849‡
1	–	–	7	
2	–	–	15	
3	–	–	10	
Tumour stage				
I	–	–	6	0.918‡
II	–	–	6	
III	–	–	16	
IV	–	–	4	
Survival (months) (mean (SD))	–	–	15.6 (8.7)	0.82†

*Relationship to VR1 mRNA expression in pancreatic cancer.
†Spearman correlation test.
‡Kruskal-Wallis test.

(cyclophilin B and hypoxanthine-guanine phosphoribosyltransferase; all primers obtained from Search-LC, Heidelberg, Germany) before graph plotting. Quantitative PCR was performed in human pancreatic cancer tissue, normal pancreatic tissue, chronic pancreatitis tissue, and pancreatic cancer cell lines (Capan-1, MIA PaCa-2).

Annexin V staining and FACS analysis

Detection of apoptosis was performed using costaining with annexin V and propidium iodide, as described previously.[46] Pancreatic tumour cells (1×10^5 cells/well) were seeded onto six well plates in 1% fetal bovine serum containing medium, allowed to adhere overnight, and then treated with the IC_{50} concentrations of gemcitabine, 5-FU, and 10 μM RTX. To analyse cell cycle distribution, cells were collected after 48 hours of incubation, washed with phosphate buffered saline, resuspended in 0.5 ml of hypotonic propidium iodide buffer (5 μg/ml propidium iodide, 0.1% Triton X100, and 0.1% sodium citrate), stored overnight at 4°C, and then analysed by flow cytometry using BD-LSR (Becton Dickinson, BD). The resulting DNA histograms were interpreted using the Cell Quest Pro software. To determine the degree of cell death, cells were collected after 24 hours of exposure to the IC_{50} of 5-FU or gemcitabine, or 10 μM RTX, washed, and stained with annexin-V-FITC (apoptotic death) or propidium iodide (necrotic death) according to the manufacturer's instructions (Roche, Mannheim, Germany).

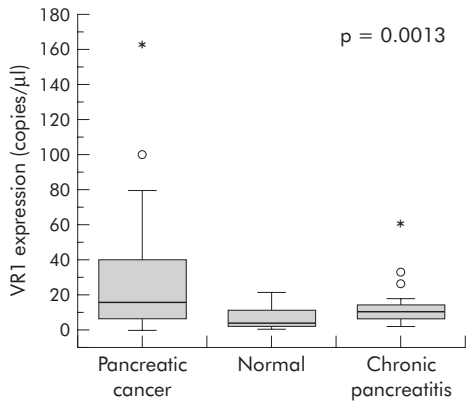

Figure 1 Vanilloid 1 receptor (VR1) expression in normal human pancreas, chronic pancreatitis, and pancreatic adenocarcinoma, shown by quantitative polymerase chain reaction.

Effect of RTX on hydrogen peroxide production in pancreatic cancer cells

Oxidation of 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), a non-fluorescent probe, to a fluorescent

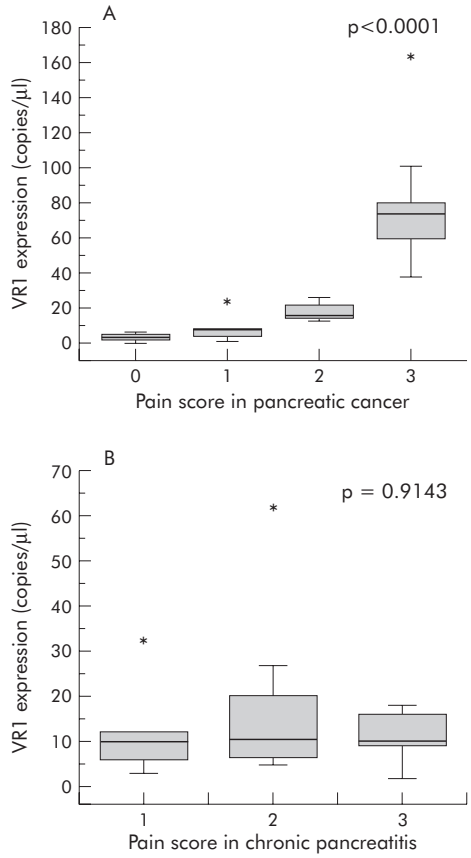

Figure 2 (A) Correlation of different pain levels with vanilloid 1 receptor (VR1) expression in patients with pancreatic cancer, presented as box and whisker plots. (B) Correlation of different pain levels with VR1 expression in patients with chronic pancreatitis, presented as box and whisker plots (see statistical analysis for details).

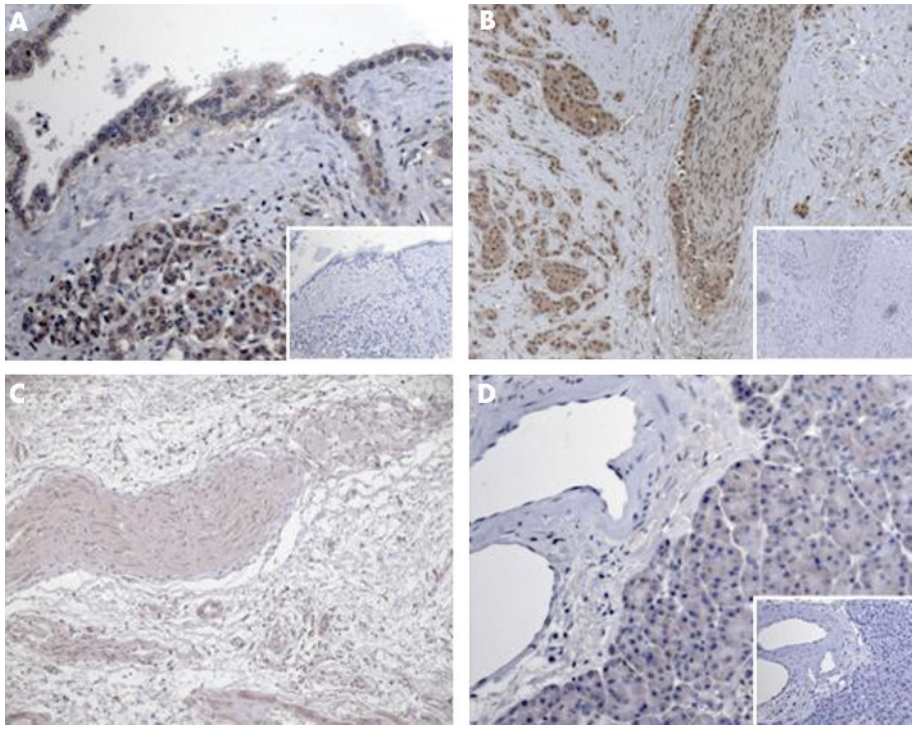

Figure 3 Immunohistochemistry with affinity purified polyclonal anti-vanilloid 1 receptor (VR1) (1:1000) in the normal human pancreas and pancreatic cancer. (A) Pancreatic ductal adenocarcinoma with VR1 staining in ductal cancer cells and acinus cells, and negative control staining. (B) Pancreatic ductal adenocarcinoma with VR1 staining in nerves infiltrated by cancer cells in patients with high pain score levels, and negative control staining. (C) Pancreatic ductal adenocarcinoma with VR1 staining in nerves located in areas of pancreatitis around the tumour. (D) Normal pancreas with VR1 staining in acinus cells, no staining in ducts or nerves, and negative control staining.

2′,7′-dichlorofluorescein (DCF) was reported to measure intracellular H_2O_2 production, as previously described by Hail and Lotan.[47]

Cells in 10 cm plastic tissue culture plates were incubated for 20 minutes with 20 μM DCFH-DA diluted in Krebs-Ringer buffer. 2′,7′-Dichlorofluorescein diacetate is cell permeant and is cleaved by esterase activity to form DCF, which is retained inside the cell. DCF can then be converted to the fluorescent product DCF in the presence of hydrogen peroxide. Cells were washed once with 5 ml of Krebs-Ringer buffer to remove the residual dye and then treated for 30 minutes with RTX or with complete medium, respectively. The green fluorescence characteristic of DCF product formation was monitored for the first 60 minutes after RTX addition using a fluorescence microscope equipped with fluorescein isothiocyanate filters, revealing a maximum of fluorescence after 30–45 minutes of RTX exposure. After treatment with RTX, cells were also harvested and analysed immediately by flow cytometry for DCF fluorescence intensity (FACS analysis). All experiments were performed in triplicate.

Statistical analysis

Statistical analysis was performed using SAS software (release 9.1; SAS Institute Inc., Cary, North Carolina, USA). Non-parametric statistical methods were used to analyse VR1 expression in subgroups of patients judged by the Shapiro-Wilk test. The distribution of VR1 expression in subgroups of patients was presented as box and whisker plots with minimum, 25th percentile, median, 75th percentile, maximum, outliers (O), and extreme values (*). Comparison between VR1 expression in patients with pancreatic cancer, chronic pancreatitis, and control patients was performed using the Kruskal-Wallis test. The association of VR1 expression and different pain levels (stage 0–3) in tumour patients was analysed using the Kruskal-Wallis test and the Mann-Whitney U test. The correlation of VR1 expression with age and survival was determined by the Spearman correlation coefficient. The Kruskal-Wallis test was performed to analyse the association of VR1 expression and tumour grading and tumour stage (UICC and TNM). The same variables have been correlated with VR1 expression regarding different groups of VR1 expression (VR1 expression <10, 10–29, $\geqslant 30$). Two sided p values were reported in all cases and an effect was considered statistically significant at a p value of $\leqslant 0.05$.

RESULTS

VR1 expression in the normal pancreas and pancreatic cancer (quantitative PCR): correlation with clinical data

VR1 mRNA expression was significantly increased in pancreatic cancer compared with the normal pancreas (normal: mean 4.0, quartiles 5.7; cancer: mean 16.0, quartiles 35.5; $p = 0.0006$) (fig 1). Analysis of VR1 expression based on clinical data showed a significant linear relationship between the pain score given by cancer patients (levels 1–3) and VR1 mRNA expression ($p<0.0001$) (fig 2A). The relationship of VR1 expression in cancer patients with pain score levels 1 and 2 differed significantly ($p = 0.024$), as much as between cancer patients with pain score levels 2 and 3 ($p = 0.0002$). There was no significant difference in VR1 expression between pain score levels 0 and 1 ($p = 0.1$). Correlation of VR1 expression with other clinical data such as age, tumour grade, tumour stage, or survival did not show any relevant relationships ($p>0.05$; table 2) in patients with pancreatic cancer. Furthermore, a relationship between clinical data and VR1 expression groups, summarised as <10, 10–29, and $\geqslant 30$ copies/μl, could not be shown (data not shown).

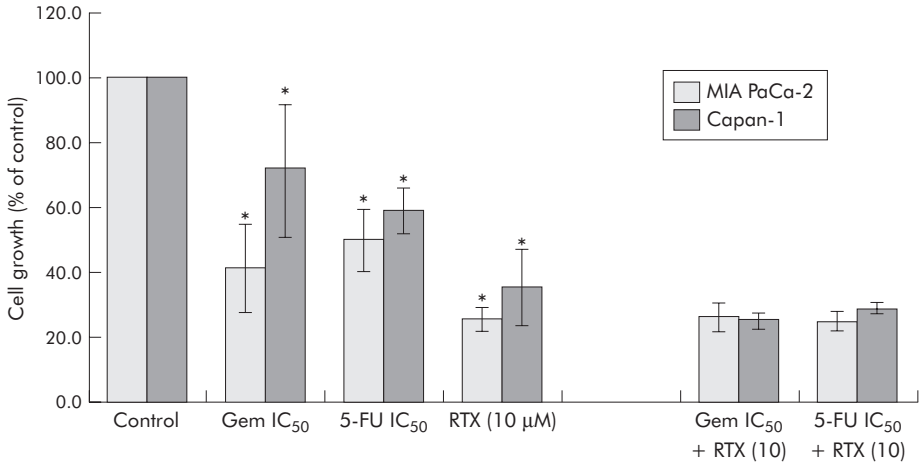

Figure 4 Tumour cell growth (MTT) in pancreatic cancer cells (MIA PaCa and Capan-1) after treatment with resiniferatoxin (RTX), 5-fluorouracil (5-FU), and gemcitabine. Measurements are as described in materials and methods.

Statistical analysis of 19 patients with chronic pancreatitis in our study showed no significant difference in vanilloid expression between patients with pancreatic cancer or chronic pancreatitis ($p = 0.17$), although there was a trend towards higher VR1 expression in pancreatic cancer (mean 29.95) than in chronic pancreatitis (mean 14.2) (fig 1). VR1 expression in patients with chronic pancreatitis was significantly higher than in donor patients with normal pancreas ($p = 0.016$). We registered no relationship between pain score level of patients with chronic pancreatitis and VR1 expression, similar to tumour patients (chi-quadrat: $p = 0.914$) (fig 2B).

Localisation of VR1 in normal pancreas and pancreatic cancer (immunohistochemistry)

Pancreatic cancer cells, ducts, acinar cells, and nerves (especially nerves in the inflamed tissue surrounding the cancer, and nerves infiltrated by pancreatic cancer cells) in human pancreatic cancer showed positive staining for VR1 whereas in normal pancreas VR1 immunoreactivity was mainly present in acinar cells, but not in ductal cells or nerves (fig 3A–D). Cancer patients with high pain score levels showed more VR1 positive stained nerves infiltrated by pancreatic cancer cells (fig 3B) than patients with low pain score levels.

Measurement of cell growth under chemotherapy with and without RTX

The pancreatic cancer cell lines MIA PaCa-2 and Capan-1 showed different cell growth rates after treatment with gemcitabine: cell growth was reduced to 40% in MIA PaCa-2, but only to 72% in Capan-1. RTX alone decreased cell growth to 25.5% in MIA PaCa-2 and to 35.3% in Capan-1. Cell growth in MIA PaCa-2 and Capan-1 lines undergoing treatment with gemcitabine in combination with RTX was almost the same as for those cell lines treated with RTX alone (26.1% *v* 25.5% ($p>0.8$) in MIA PaCa-2; 24.9% *v* 35.3% ($p>0.5$) in Capan-1) (fig 4).

The two cell lines (MIA PaCa-2 and Capan-1) treated with 5-FU showed similar results for cell growth as those treated with gemcitabine (fig 4). Cell growth was reduced to 49.9% in MIA PaCa and to only 59% in Capan-1 treated with 5-FU; in MIA PaCa-2 and Capan-1 lines undergoing treatment with 5-FU in combination with RTX, cell growth was again almost the same as in lines treated with RTX alone (24.7% *v* 25.5% ($p>0.8$) in MIA PaCa-2; 28.7% *v* 35.3% in Capan-1 ($p>0.5$)). The same results, especially regarding no additive effect of the drugs, were obtained with reduced concentrations of the chemotherapeutic drugs (one fifth of the IC_{50}) and RTX (2 μM) (data not shown).

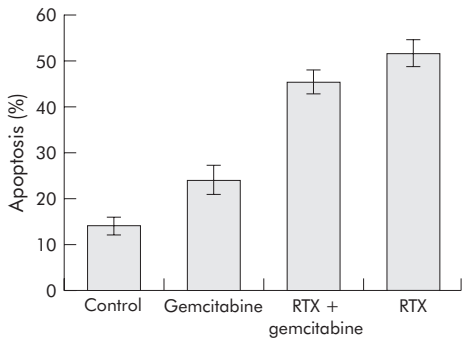

Figure 5 Apoptotic cells in MIA PaCa-2 treated with resiniferatoxin (RTX) and gemcitabine (right upper and lower quadrant—see fig 6). Mean of three independent FACS analyses, as described in materials and methods.

Apoptosis measurement by FACS analysis under chemotherapy with and without RTX

A 24 hour exposure to 10 μM RTX was sufficient to promote apoptosis in 51.5% of MIA PaCa-2 samples (data for 2 μM and 100 μM not shown). This concentration of RTX chosen for our experiments is in agreement with that of previous reports.[13 20 48] In both pancreatic cancer cell lines undergoing chemotherapy with 5-FU or gemcitabine or a combination of chemotherapy with RTX, increasing apoptosis was observed compared with untreated pancreatic cancer cells (data shown for MIA PaCa-2 and 10 μM RTX, gemcitabine, or gemcitabine + 10 μM RTX; figs 5, 6). The strongest effect on apoptosis was seen in MIA PaCa-2 cancer cells treated with 10 μM RTX alone. An additive effect on apoptosis of either 5-FU or gemcitabine together with 10 μM RTX could not be confirmed. The differences in effect on apoptosis between the groups (gemcitabine alone, RTX + gemcitabine, and RTX alone) were significant ($p<0.05$).

Measurement of oxidative stress in pancreatic cancer cells

Results in both cell lines showed that RTX at a concentration of 10 μM induced an increase in DCF staining 15 minutes after exposure, reaching a maximum at 30–60 minutes compared with the group without RTX treatment showing only weak staining (data shown for MIA PaCa-2; fig 7B). Three independent experiments were performed. These results suggest that RTX promotes intracellular oxidant

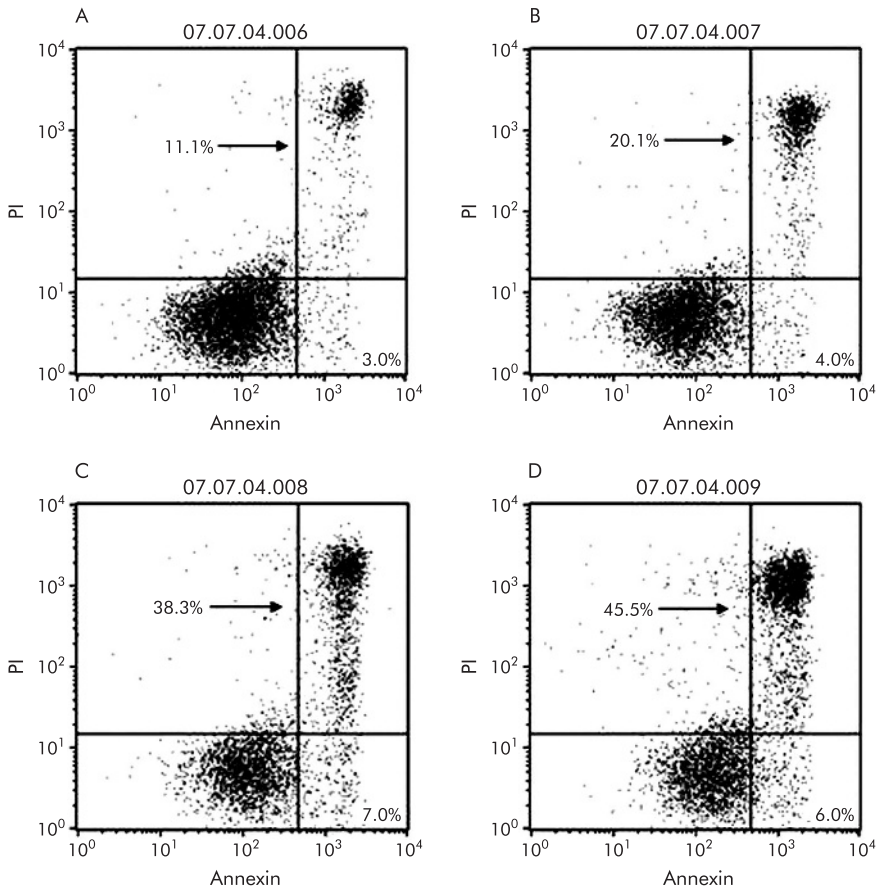

Figure 6 Exemplary FACS analysis in pancreatic cancer cells (MIA PaCa-2) treated for 24 hours with gemcitabine (IC_{50}), 5-fluorouracil (IC_{50}), and resiniferatoxin (10 μM): (A) Control group (14.1% apoptotic cells). (B) Gemcitabine treatment (24.1% apoptotic cells). (C) Gemcitabine + resiniferatoxin treatment (45.3% apoptotic cells). (D) Resiniferatoxin treatment (51.5% apoptotic cells). PI, propidium iodide.

generation, as detected by DCF fluorescence. Enhanced hydrogen peroxide generation before induction of mitochondrial permeability transition by RTX can be explained by vanilloid induced inhibition of mitochondrial electron transport promoting the production of non-enzymatic reactive oxygen species due to redox cycling of reduced electron carriers upstream from the site of inhibition.[13 48 49] Vanilloid exposure resulted in a peak of DCF at approximately 45 minutes and revealed at that time a 6.2-fold increased mean DCF fluorescence over controls (FACS analysis in MIA PaCa-2; fig 7A).

DISCUSSION

In our present study, RTX induced apoptosis and inhibited pancreatic cancer cell growth, effects which seemed to be mediated by mitochondrial dysregulation independent of VR1. VR1 was upregulated at the mRNA and protein levels in human pancreatic cancer and chronic pancreatitis in comparison with patients with a normal pancreas. Immunoreactivity of VR1 in tumour infiltrated or inflamed nerves closely matched elevated mRNA expression, which was related to the intensity of pain in pancreatic cancer patients but not in chronic pancreatitis.

The vanilloid receptor seems to play no role in vanilloid induced apoptosis in VR1 expressing pancreatic cancer cells. Corresponding results suggest that apoptosis in human glioblastoma cells did not appear to be mediated by VR1 receptors, because capsazepine (prototypical vanilloid receptor antagonist) was unable to block this effect.[12] Our study supports a VR1 independent effect of vanilloids acting as coenzyme Q analogues, blocking the electron transfer at the NADH:coenzyme Q oxidoreductase of the mitochondrial electron transport chain, as indicated by higher production of hydrogen peroxide in our experiment.[21–24] We found that exposure of MIA PaCa-2 to RTX was sufficient to promote an approximately 6.2-fold increase in mean production of hydrogen peroxide compared with that produced by the control group, which was stronger than in the literature under comparable conditions.[9]

The question of whether capsaicin and RTX trigger oxidative stress and apoptosis in SCC cells by blocking plasma membrane NADH oxidoreductase electron transport chain activity, complex I activity, or both, was recently addressed by Hail and Lotan using respiration deficient clones lacking mitochondrial DNA derived from COLO 16 and SRB-12 cells.[9] Hail's data indicated that most of the initial hydrogen peroxide produced in COLO 16 and SRB-12 cells exposed to vanilloids was of mitochondrial origin and was probably associated with inhibition of mitochondrial respiration.

As these results were not related to the sensitivity of cancer cells towards RTX induced cytotoxicity, we addressed this question in our experiments, performing both apoptosis and cell growth tests with human pancreatic cancer cell lines. RTX induced cytotoxicity in pancreatic cancer cell lines was demonstrated by a significant increase in apoptosis and by a decrease in cell growth. For the first time we demonstrated the inhibiting effect on mitochondrial respiration by RTX together with its cytotoxic effect in pancreatic cancer cells.

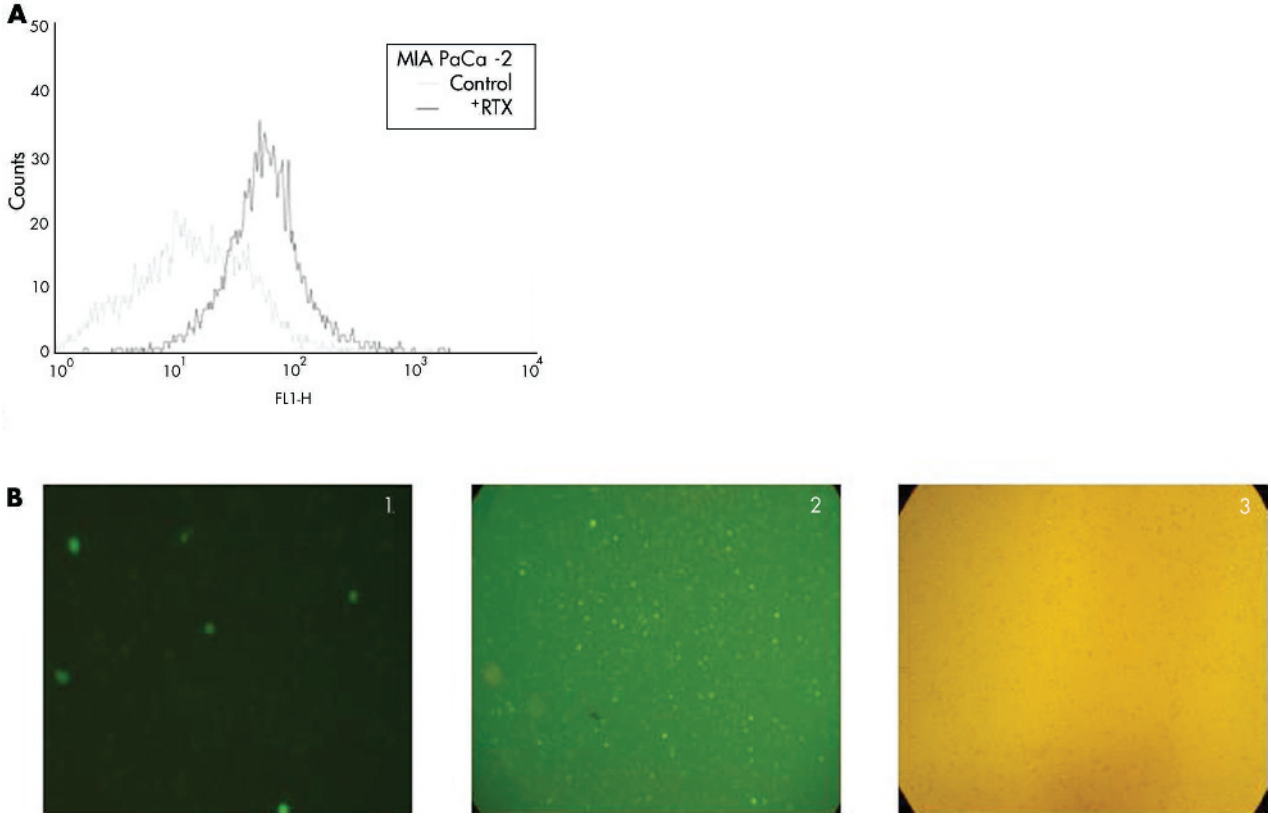

Figure 7 Measurement of oxidative stress by FACS analysis in pancreatic cancer cells after treatment with resiniferatoxin (RTX) using dichlorodihydrofluorescein probes. (A) Quantification of hydroperoxide production in resiniferatoxin treated MIA PaCa-2 cells with the use of 2′,7′-dichlorofluorescin (DCF) by FACS analysis (exemplary histogram). X axis = DCF fluorescence intensity; y axis = relative frequency of cells (count) exhibiting varying degrees of DCF fluorescence. (B1) MIA PaCa-2 without RTX showing limited staining of fluorescent dichlorofluorescein; (B2) MIA PaCa-2 with 10 μM RTX induced an increase in fluorescent dichlorofluorescein staining, reaching a maximum at 30–45 minutes in MIA PaCa-2; (B3) MIA PaCa-2 without RTX and fluorescent DCF (control group).

Until now, chemotherapy has had limited efficacy, and improvement in survival has been unsatisfactory in patients with pancreatic cancer.[4] Our in vitro results indicate a strong effect of RTX on apoptosis induction and a reduction in cell growth. As the efficacy of this treatment could not be increased by gemcitabine or 5-FU, it seems that both strategies have the same target. Indeed, like RTX, 5-FU and gemcitabine can activate mitochondria mediated apoptosis through the caspase cascade.[50 51]

Based on higher expression of VR1 in pancreatic cancer cells and nerves compared with the normal pancreas, along with the result that VR1-RNA expression is related to increasing pain levels in cancer patients, vanilloids such as RTX appear to be useful in managing certain types of neurogenic pain and inflammation. The effect of vanilloids on the vanilloid receptor shown in primary afferent nerves is excitation followed by desensitisation due to elevations in intracellular free Ca^{2+}.[36] Once vanilloids diffuse across the plasma membrane and bind to the cytoplasmic domains of vanilloid receptors, the receptors can become active action channels.[52 53] Excessive Ca^{2+} influx triggered by vanilloid exposure can disrupt Ca^{2+} homeostasis and promote excitotoxicity in nociceptive neurones.[33 40 54–56] Capsaicin and RTX either desensitise the nociceptive nerve ending or completely delete the neurone itself.[26 35 55] Enhancement of vanilloid sensitivity can be mediated by nerve growth factor and by proinflammatory cytokines such as tumour necrosis factor α or interleukin 1β.[57 58] Furthermore, protons reducing the extracellular pH to levels below the physiological norm of about 7.6 have been shown to act as modulators of native vanilloid receptors.[40 59]

In inflammatory bowel disease, VR1-like immunoreactivity is substantially higher in nerve fibres compared with the normal bowel.[60] As cancer cell proliferation is also associated with tissue damage (inflammation and ischaemia), producing a number of chemical mediators that activate or sensitise nociceptor terminals to elicit pain (protons), our data indicate that VR1 may play a role in pain generation in pancreatic cancer patients. In vivo treatment with RTX blocks experimental inflammatory hyperalgesia and neurogenic inflammation naturally occurring in rats with cancer, as well as blocking debilitating arthritic pain in dogs. In contrast, sensations of touch, proprioception, and high threshold mechanosensitive nociception, as well as locomotor function, remain intact.[61] These experimental data are in line with observations in humans in whom RTX application in doses between 0.01 μM and 0.1 μM significantly reduced bladder hypersensitivity.[62]

These findings, in combination with the results of our study, show that nociceptive neuronal or nerve terminal deletion by RTX could be effective and broadly applicable as strategies for pain management in patients with pancreatic cancer. Concordant with the relationship between VR1 expression and pain score levels demonstrated in pancreatic cancer patients (but no relationship between VR1 expression and tumour stage or grading), we found pronounced nerve infiltration of pancreatic cancer cells in patients with higher pain score levels demonstrated immunohistochemically by

VR1 expression. Whether tumour infiltration of nerves alone or, apart from this, the VR1 positive inflamed nerves around the cancer tissue, is responsible for the relationship between VR1 expression and pain score levels in pancreatic cancer patients, is the goal of further investigations. As we could not demonstrate a relationship between pain score levels and VR1 expression in patients with chronic pancreatitis, these questions may only be answered by extended evaluation of a larger number of patients with pancreatic cancer or chronic pancreatitis and functional experiments.

In conclusion, RTX induces apoptosis by targeting mitochondrial respiration in pancreatic cancer cells, suggesting that it might be useful in the prevention or treatment of pancreatic cancer. Furthermore, we have shown that the vanilloid receptor is significantly upregulated in human pancreatic cancer tissue, and its level of expression is related to the intensity of pain reported by patients. Therefore, together with results from the literature proposing RTX induced Ca^{2+} cytotoxicity as the mechanism for therapeutic neurone deletion, our data suggest that vanilloids such as RTX might be novel and effective treatment options for neurogenic cancer pain.

Authors' affiliations

M Hartel, F F di Mola, F Selvaggi, G Mascetta, M N Wente, K Felix, N A Giese, U Hinz, P D Sebastiano, M W Büchler, H Friess, Department of General Surgery, University of Heidelberg, Heidelberg, Germany

Conflict of interest: None declared.

REFERENCES

1 **Jemal A**, Tiwari RC, Murray T, *et al*. Cancer statistics, 2004. *CA Cancer J Clin* 2004;**54**:8–29.
2 **Hoffman JP**, Lipsitz S, Pisansky T, *et al*. Phase II trial of preoperative radiation therapy and chemotherapy for patients with localized, resectable adenocarcinoma of the pancreas: an Eastern Cooperative Oncology Group Study. *J Clin Oncol* 1998;**16**:317–23.
3 **Neoptolemos JP**, Cunningham D, Friess H, *et al*. Adjuvant therapy in pancreatic cancer: historical and current perspectives. *Ann Oncol* 2003;**14**:675–92.
4 **Neoptolemos JP**, Stocken DD, Friess H, *et al*. A randomized trial of chemoradiotherapy and chemotherapy after resection of pancreatic cancer. *N Engl J Med* 2004;**350**:1200–10.
5 **Pendurthi TK**, Hoffman JP, Ross E, *et al*. Preoperative versus postoperative chemoradiation for patients with resected pancreatic adenocarcinoma. *Am Surg* 1998;**64**:686–92.
6 **Spitz FR**, Abbruzzese JL, Lee JE, *et al*. Preoperative and postoperative chemoradiation strategies in patients treated with pancreaticoduodenectomy for adenocarcinoma of the pancreas. *J Clin Oncol* 1997;**15**:928–37.
7 **Fesus L**, Szondy Z, Uray I. Probing the molecular program of apoptosis by cancer chemopreventive agents. *J Cell Biochem Suppl* 1995;**22**:151–61.
8 **Sharma RA**, Manson MM, Gescher A, *et al*. Colorectal cancer chemoprevention: biochemical targets and clinical development of promising agents. *Eur J Cancer* 2001;**37**:12–22.
9 **Hail N Jr**, Lotan R. Examining the role of mitochondrial respiration in vanilloid-induced apoptosis. *J Natl Cancer Inst* 2002;**94**:1281–92.
10 **Jung MY**, Kang HJ, Moon A. Capsaicin-induced apoptosis in SK-Hep-1 hepatocarcinoma cells involves Bcl-2 downregulation and caspase-3 activation. *Cancer Lett* 2001;**165**:139–45.
11 **Kim JD**, Kim JM, Pyo JO, *et al*. Capsaicin can alter the expression of tumor forming-related genes which might be followed by induction of apoptosis of a Korean stomach cancer cell line, SNU-1. *Cancer Lett* 1997;**120**:235–41.
12 **Lee YS**, Nam DH, Kim JA. Induction of apoptosis by capsaicin in A172 human glioblastoma cells. *Cancer Lett* 2000;**161**:121–30.
13 **Macho A**, Lucena C, Calzado MA, *et al*. Phorboid 20-homovanillates induce apoptosis through a VR1-independent mechanism. *Chem Biol* 2000;**7**:483–92.
14 **Morre DJ**, Sun E, Geilen C, *et al*. Capsaicin inhibits plasma membrane NADH oxidase and growth of human and mouse melanoma lines. *Eur J Cancer* 1996;**32A**:1995–2003.
15 **Tanaka T**, Kohno H, Sakata K, *et al*. Modifying effects of dietary capsaicin and rotenone on 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis. *Carcinogenesis* 2002;**23**:1361–7.
16 **Wolvetang EJ**, Larm JA, Moutsoulas P, *et al*. Apoptosis induced by inhibitors of the plasma membrane NADH-oxidase involves Bcl-2 and calcineurin. *Cell Growth Differ* 1996;**7**:1315–25.
17 **Jang JJ**, Kim SH, Yun TK. Inhibitory effect of capsaicin on mouse lung tumor development. *In Vivo* 1989;**3**:49–53.
18 **Park KK**, Surh YJ. Effects of capsaicin on chemically-induced two-stage mouse skin carcinogenesis. *Cancer Lett* 1997;**114**:183–4.
19 **Surh YJ**, Lee RC, Park KK, *et al*. Chemoprotective effects of capsaicin and diallyl sulfide against mutagenesis or tumorigenesis by vinyl carbamate and N-nitrosodimethylamine. *Carcinogenesis* 1995;**16**:2467–71.
20 **Morre DJ**, Chueh PJ, Morre DM. Capsaicin inhibits preferentially the NADH oxidase and growth of transformed cells in culture. *Proc Natl Acad Sci U S A* 1995;**92**:1831–5.
21 **Chudapongse P**, Janthasoot W. Mechanism of the inhibitory action of capsaicin on energy metabolism by rat liver mitochondria. *Biochem Pharmacol* 1981;**30**:735–40.
22 **Okun JG**, Lummen P, Brandt U. Three classes of inhibitors share a common binding domain in mitochondrial complex I (NADH:ubiquinone oxidoreductase). *J Biol Chem* 1999;**274**:2625–30.
23 **Shimomura Y**, Kawada T, Suzuki M. Capsaicin and its analogs inhibit the activity of NADH-coenzyme Q oxidoreductase of the mitochondrial respiratory chain. *Arch Biochem Biophys* 1989;**270**:573–7.
24 **Yagi T**. Inhibition by capsaicin of NADH-quinone oxidoreductases is correlated with the presence of energy-coupling site 1 in various organisms. *Arch Biochem Biophys* 1990;**281**:305–11.
25 **Kroemer G**, Petit P, Zamzami N, *et al*. The biochemistry of programmed cell death. *FASEB J* 1995;**9**:1277–87.
26 **Olah Z**, Szabo T, Karai L, *et al*. Ligand-induced dynamic membrane changes and cell deletion conferred by vanilloid receptor 1. *J Biol Chem* 2001;**276**:11021–30.
27 **Santoni G**, Perfumi MC, Pompei P, *et al*. Impairment of rat thymocyte differentiation and functions by neonatal capsaicin treatment is associated with induction of apoptosis. *J Neuroimmunol* 2000;**104**:37–46.
28 **Huang MT**, Ma W, Yen P, *et al*. Inhibitory effects of topical application of low doses of curcumin on 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion and oxidized DNA bases in mouse epidermis. *Carcinogenesis* 1997;**18**:83–8.
29 **Limtrakul P**, Lipigorngoson S, Namwong O, *et al*. Inhibitory effect of dietary curcumin on skin carcinogenesis in mice. *Cancer Lett* 1997;**116**:197–203.
30 **Samaha HS**, Kelloff GJ, Steele V, *et al*. Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate, and 6-phenylhexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. *Cancer Res* 1997;**57**:1301–5.
31 **Costantini P**, Jacotot E, Decaudin D, *et al*. Mitochondrion as a novel target of anticancer chemotherapy. *J Natl Cancer Inst* 2000;**92**:1042–53.
32 **Lee E**, Surh YJ. Induction of apoptosis in HL-60 cells by pungent vanilloids, [6]-gingerol and [6]-paradol. *Cancer Lett* 1998;**134**:163–8.
33 **Dedov VN**, Mandadi S, Armati PJ, *et al*. Capsaicin-induced depolarisation of mitochondria in dorsal root ganglion neurons is enhanced by vanilloid receptors. *Neuroscience* 2001;**103**:219–26.
34 **Hiura A**, Nakae Y, Nakagawa H. Cell death of primary afferent nerve cells in neonatal mice treated with capsaicin. *Anat Sci Int* 2002;**77**:47–50.
35 **Jancso G**, Kiraly E, Jancso-Gabor A. Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. *Nature* 1977;**270**:741–3.
36 **Szallasi A**, Blumberg PM. Vanilloid (Capsaicin) receptors and mechanisms. *Pharmacol Rev* 1999;**51**:159–212.
37 **Sugimoto T**, Takeyama A, Xiao C, *et al*. Electron microscopic demonstration of nick end-labeled DNA fragments during capsaicin-induced apoptosis of trigeminal primary neurons in neonatal rats. *Brain Res* 1999;**818**:147–52.
38 **Sugimoto T**, Xiao C, Ichikawa H. Neonatal primary neuronal death induced by capsaicin and axotomy involves an apoptotic mechanism. *Brain Res* 1998;**807**:147–54.
39 **Bernabei R**, Gambassi G, Lapane K, *et al*. Management of pain in elderly patients with cancer. SAGE Study Group. Systematic assessment of geriatric drug use via epidemiology. *JAMA* 1998;**279**:1877–82.
40 **Caterina MJ**, Schumacher MA, Tominaga M, *et al*. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997;**389**:816–24.
41 **Cleeland CS**, Gonin R, Hatfield AK, *et al*. Pain and its treatment in outpatients with metastatic cancer. *N Engl J Med* 1994;**330**:592–6.
42 **Wolfe J**, Grier HE, Klar N, *et al*. Symptoms and suffering at the end of life in children with cancer. *N Engl J Med* 2000;**342**:326–33.
43 **Kleeff J**, Ishiwata T, Kumbasar A, *et al*. The cell-surface heparan sulfate proteoglycan glypican-1 regulates growth factor action in pancreatic carcinoma cells and is overexpressed in human pancreatic cancer. *J Clin Invest* 1998;**102**:1662–73.
44 **Erkan M**, Kleeff J, Esposito I, *et al*. Loss of BNIP3 expression is a late event in pancreatic cancer contributing to chemoresistance and worsened prognosis. *Oncogene* 2005;**24**:4421–32.
45 **Li J**, Kleeff J, Guweidhi A, *et al*. RUNX3 expression in primary and metastatic pancreatic cancer. *J Clin Pathol* 2004;**57**:294–9.
46 **Li J**, Kleeff J, Giese N, *et al*. Gefitinib ('Iressa', ZD1839), a selective epidermal growth factor receptor tyrosine kinase inhibitor, inhibits pancreatic cancer cell growth, invasion, and colony formation. *Int J Oncol* 2004;**25**:203–10.
47 **Hail N Jr**, Lotan R. Mitochondrial respiration is uniquely associated with the prooxidant and apoptotic effects of N-(4-hydroxyphenyl)retinamide. *J Biol Chem* 2001;**276**:45614–21.
48 **Macho A**, Blazquez MV, Navas P, *et al*. Induction of apoptosis by vanilloid compounds does not require de novo gene transcription and activator protein 1 activity. *Cell Growth Differ* 1998;**9**:277–86.
49 **Macho A**, Calzado MA, Munoz-Blanco J, *et al*. Selective induction of apoptosis by capsaicin in transformed cells: the role of reactive oxygen species and calcium. *Cell Death Differ* 1999;**6**:155–65.
50 **Longley DB**, Allen WL, McDermott U, *et al*. The roles of thymidylate synthase and p53 in regulating Fas-mediated apoptosis in response to antimetabolites. *Clin Cancer Res* 2004;**10**:3562–71.

51 **Schniewind B**, Christgen M, Kurdow R, *et al*. Resistance of pancreatic cancer to gemcitabine treatment is dependent on mitochondria-mediated apoptosis. *Int J Cancer* 2004;**109**:182–8.
52 **Jung J**, Hwang SW, Kwak J, *et al*. Capsaicin binds to the intracellular domain of the capsaicin-activated ion channel. *J Neurosci* 1999;**19**:529–38.
53 **Jung J**, Lee SY, Hwang SW, *et al*. Agonist recognition sites in the cytosolic tails of vanilloid receptor 1. *J Biol Chem* 2002;**277**:44448–54.
54 **Chard PS**, Bleakman D, Savidge JR, *et al*. Capsaicin-induced neurotoxicity in cultured dorsal root ganglion neurons: involvement of calcium-activated proteases. *Neuroscience* 1995;**65**:1099–108.
55 **Winter J**, Dray A, Wood JN, *et al*. Cellular mechanism of action of resiniferatoxin: a potent sensory neuron excitotoxin. *Brain Res* 1990;**520**:131–40.
56 **Wood JN**, Winter J, James IF, *et al*. Capsaicin-induced ion fluxes in dorsal root ganglion cells in culture. *J Neurosci* 1988;**8**:3208–20.
57 **Anand P**, Terenghi G, Warner G, *et al*. The role of endogenous nerve growth factor in human diabetic neuropathy. *Nat Med* 1996;**2**:703–7.
58 **Nicol GD**, Lopshire JC, Pafford CM. Tumor necrosis factor enhances the capsaicin sensitivity of rat sensory neurons. *J Neurosci* 1997;**17**:975–82.
59 **Jordt SE**, Tominaga M, Julius D. Acid potentiation of the capsaicin receptor determined by a key extracellular site. *Proc Natl Acad Sci U S A* 2000;**97**:8134–9.
60 **Yiangou Y**, Facer P, Dyer NH, *et al*. Vanilloid receptor 1 immunoreactivity in inflamed human bowel. *Lancet* 2001;**357**:1338–9.
61 **Karai L**, Brown DC, Mannes AJ, *et al*. Deletion of vanilloid receptor 1-expressing primary afferent neurons for pain control. *J Clin Invest* 2004;**113**:1344–52.
62 **Payne CK**, Mosbaugh PG, Forrest JB, *et al*. Intravesical resiniferatoxin for the treatment of interstitial cystitis: a randomized, double-blind, placebo-controlled trial. *J Urol* 2005;**173**:1590–4.

EDITOR'S QUIZ: GI SNAPSHOT

Answer

From question on page 477

Contrast enhanced computed tomography scan showed a large hypoattenuating hepatic cystic lesion with intralesional air fluid levels (fig 2, white arrow) and intraperitoneal free air accumulation (fig 2, black arrows). The lesion represented a ruptured gas forming pyogenic liver abscess (GFPLA). The patient fell into hypotension in the emergency department and underwent emergency laparoscopic abscess drainage with peritoneal lavage (fig 3). Approximately 30 ml of greenish muddy appearing fluid were obtained, which yielded *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Enterococcus* spp in bacterial culture. One day later, the patient's fever abated, and the white cell count returned rapidly to normal. Sonographic scan six months later revealed disappearance of the liver lesion.

Ten to twenty per cent of patients with liver abscess may develop GFPLA, which is frequently associated with a higher incidence of complications and mortality. *K pneumoniae* infection in diabetic patients is the cornerstone for the development of GFPLA. Gas in the abscess has been suggested to result from glucose fermentation by *K pneumonia*. Approximately 7.1–15.1% of cases may experience rupture of a GFPLA due to intra-abscess air pressure overload. When this occurs, the clinical picture mimics hollow organ perforation and poses a diagnostic challenge.

Prompt percutaneous drainage and antibiotic treatment have been shown to reduce mortality in uncomplicated cases. Open surgery is advocated for ruptured pyogenic liver abscesses with secondary peritonitis. Efficacy of laparoscopic drainage has been proved recently, which carries the benefits of minimally invasive surgery.

doi: 10.1136/gut.2005.076091

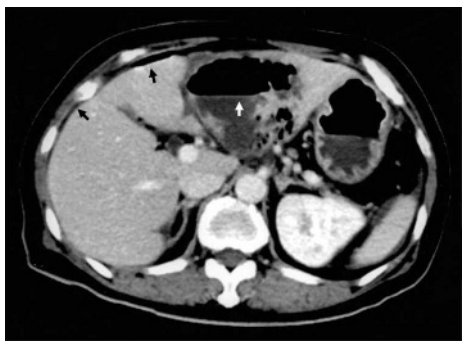

Figure 2 Contrast enhanced computed tomography scan showed a large hypoattenuating hepatic cystic lesion with intralesional air fluid levels (white arrow) and intraperitoneal free air accumulation (black arrows).

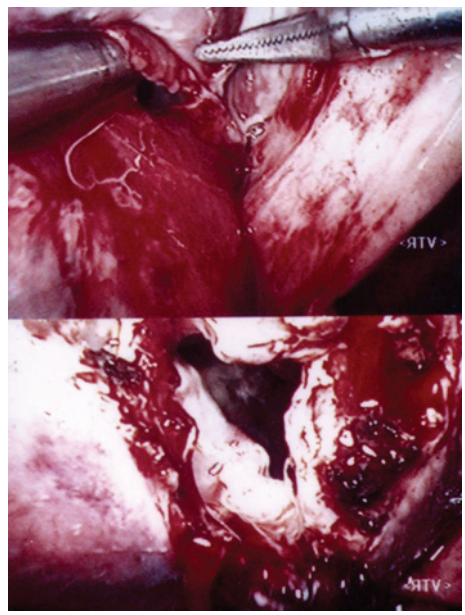

Figure 3 The patient underwent laparoscopic fenestration and abscess drainage with peritoneal lavage.