

PANCREATITIS

Anti-monocyte chemoattractant protein 1 gene therapy attenuates experimental chronic pancreatitis induced by dibutyltin dichloride in rats

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Background: Monocyte chemoattractant protein 1 (MCP-1) is a member of the C-C chemokine family and exerts strong chemoattractant activity in monocytes, macrophages, and lymphocytes. Rat pancreatic fibrosis induced by dibutyltin dichloride (DBTC) is considered to be an appropriate chronic pancreatitis model histologically and enzymatically, as has demonstrated in a previous study.

Aim: We examined the effect of human dominant negative inhibitor of MCP-1 (mutant MCP-1) on progression of chronic pancreatitis induced by DBTC in a rat model.

Methods: We used the experimental model of chronic pancreatitis induced by DBTC in rats. Mutant MCP-1 or empty plasmid at a dose of 50 µg/body weight was administered into rat thigh muscles on days 4, 11, and 18 after administration of DBTC. On days 14 and 28, we evaluated the effect of mutant MCP-1 morphologically and biochemically.

Results: The mutant MCP-1 treated group inhibited early pancreatic inflammation and later pancreatic fibrosis histologically, and showed a decrease in serum MCP-1 concentration, intrapancreatic hydroxyproline, α -smooth muscle actin, and an increase in intrapancreatic amylase and protein content compared with the empty plasmid treated group. The mutant MCP-1 group also inhibited intrapancreatic mRNA expression of cytokines and chemokines.

Conclusions: Our findings suggest that monocyte/macrophage recruitment and the systemic MCP-1 signal pathway contribute to progression of chronic pancreatitis, and that blockade of MCP-1 may suppress the development of pancreatic fibrosis.

Chronic pancreatitis is an intractable disease characterised by irreversible irregular scarring of exocrine parenchyma with ductal changes subsequent to interstitial fibrosis and inflammatory processes in the pancreas.¹ However, the mechanism by which this process occurs is not fully understood. Chemokines are divided into C-X-C families and C-C families, involved in the pathogenesis of inflammatory diseases, having characteristic infiltrates. Monocyte chemoattractant protein 1 (MCP-1) is a basic protein consisting of 76 amino acids and is classified as a C-C subfamily of chemokines. It exerts strong chemoattractant activity in monocytes, macrophages, and lymphocytes. MCP-1 shows chemotaxis specific to the monocyte and is related to the manifestation of desmosomal molecules on the surface of monocytes at the inflammation site, conjugation with endothelial cells, and exudation to the subendothelium.^{2–4}

Recently, Merkord *et al* reported on acute interstitial pancreatitis in the early phase and showed that pancreatic fibrosis can be induced by dibutyltin dichloride (DBTC) in rats.^{5–6} Expression of MCP-1 was observed in pancreatic tissues from patients with chronic pancreatitis⁷ and in experimental acute pancreatitis in rats and mice.^{8–9} In our study, we demonstrated that the experimental model of pancreatic fibrosis induced by DBTC in rats was useful as a chronic pancreatitis model and that MCP-1 may play an important role in the development of pancreatic fibrosis.¹⁰

In the present study, we evaluated the use of gene therapy to block MCP-1 activity *in vivo* using an amino terminal deletion of mutant MCP-1 (mMCP-1), which lacks the terminal amino acids 2–8 and works as a potent dominant negative MCP-1 agonist.^{11–12} A previous study showed that

skeletal cells infected with mMCP-1 secrete mMCP-1 protein into circulating blood and that mMCP-1 protein competitively binds to the receptor for MCP-1 (C-C chemokine receptor (CCR-2)) on monocytes or target cells in remote organs, thus blocking the MCP-1 signal and suppressing MCP-1 mediated inflammation. This results in an improvement in the function of the target organ.¹³ Therefore, the aim of our study was to examine the effect of dominant negative inhibitor of MCP-1 (mMCP-1) on progression of the chronic pancreatitis model induced by DBTC in rats.

MATERIALS AND METHODS

Expression vector

The mMCP-1 gene was constructed by recombinant polymerase chain reaction (PCR) using a wide type human MCP-1 cDNA as a template and cloned into BamHI (5') and NotI (3') sites of the eukaryotic expression vector plasmid cDNA3 (Invitrogen Corp, Carlsbad, California, USA) as reported previously.¹⁴

Animals and experimental protocol

Adult male Lewis rats (KBT Oriental, Saga, Japan) weighing 180–200 g were used. They were maintained in accordance with the guidelines of the Committee on Animal Care of

Abbreviations: MCP-1, monocyte chemoattractant protein 1; mMCP-1, mutant MCP-1; DBTC, dibutyltin dichloride; CCR-2, C-C chemokine receptor; PSCs, pancreatic stellate cells; α -SMA, α smooth muscle actin; RT-PCR, reverse transcription-polymerase chain reaction; TGF- β , transforming growth factor β ; PDGF, platelet derived growth factor; IL-1 β , interleukin 1 β ; IL-6, interleukin 6; GAPDH, glyceraldehyde phosphate dehydrogenase; SDS, sodium dodecyl sulphate

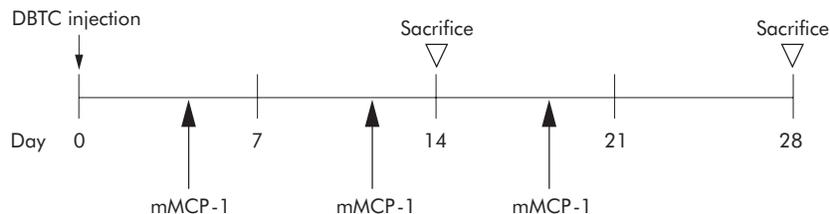


Figure 1 Experimental protocol. The dibutyltin dichloride (DBTC) solution was injected at a dose of 7 mg/kg body weight on day 0. Mutant monocyte chemoattractant protein 1 (mMCP-1) or empty plasmid (pcDNA3) was injected at a dose of 50 μ g/body into the thigh muscles on days 4, 11, and 18 after DBTC injury. Five rats from each group were sacrificed on days 14 and 28. The effect of mMCP-1 gene transfer was evaluated morphologically and biochemically.

Kyushu University. The experimental model of chronic pancreatitis was induced by a single intravenous administration of DBTC (Schering AG, Berlin, Germany), as described previously.¹⁰ Four days after DBTC injection, these rats were randomly divided into two groups and animals then received an intramuscular injection of mMCP-1 or empty plasmid. The experimental protocol is summarised in fig 1. Each rat was sacrificed at indicated days. Blood samples were collected to measure serum MCP-1 levels. Each pancreas was quickly removed and weighed. A part (tail) of each pancreas was used for histopathological analysis (haematoxylin-eosin staining, azan staining, and immunostaining for α smooth muscle actin (α -SMA)). The remaining part of each pancreas was divided and one part was homogenised in 9 volumes of ice cold buffer (50 mM Tris HCl buffer, pH 8.0, 0.5% Triton X-100), as described previously.¹⁰ The homogenates were centrifuged at 12 000 *g* for 20 minutes at 4°C. The supernatants were used for assay of amylase and protein concentrations. Another part was used for reverse transcription (RT)-PCR or western blotting. A third part of the pancreas was homogenised in 9 volumes of ice cold saline used for the hydroxyproline assay.

Chemical analysis

Amylase was measured by the Phadebas amylase test (Pharmacia Laboratories, Piscataway, NJ, USA).¹⁵ Protein content was determined by the method of Lowry and colleagues.¹⁶ Hydroxyproline was determined by a modified method according to Weidenbach and colleagues.¹⁷ Assay of endogenous MCP-1 was measured using a rat MCP-1 ELISA kit (Biosource), and mMCP-1 was measured using human specific ELISA kit (Biosource, Camarillo, California, USA), according to the manufacturer's instructions. Cross reaction of each species was less than 5% (data not shown).

RT-PCR

Total RNA was extracted from pancreatic tissue by the Isogen (Nippon Gene, Tokyo, Japan) method, as described previously.¹⁸ First standard DNA was synthesised using reverse transcriptase (River Tra Ace- Toyobo, Tokyo, Japan) according to the manufacturer's protocol. PCR primers and conditions are listed in table 1. PCR products were detected as a single band of the expected size in each PCR. The intensity of the bands was analysed using the image analysis software NIH image version 1.62 (National Institutes of Health, Bethesda, USA).

Western blot analysis for α -SMA

Pancreatic tissue was homogenated in sodium dodecyl sulphate (SDS) lysis buffer (50 mM Tris HCl, pH 7.5, 2% SDS, trypsin inhibitor at 0.2 mM), sonicated for 10 seconds, boiled for five minutes, and centrifuged at 10 000 rpm for 15 minutes. Equal amounts of protein (10 μ g) were electrophoresed through 12.5% SDS polyacrylamide gel (Bio-Rad, California, USA) and transferred to a Hybond-p (Amersham Biosciences Inc., Piscataway, New Jersey, USA). The membrane was incubated with primary antibody against α -SMA (Sigma-Aldrich, St Louis, Missouri, USA) at a dilution of 1:2000 or antibody against tubulin (Sigma-Aldrich, St Louis, Missouri, USA) at a dilution of 1:400, at 4°C overnight. After incubation with horseradish peroxidase labelled biotin rabbit antimouse IgG (Zymed, California, USA) for one hour, proteins were visualised using an ECL kit (Amersham Biosciences Co., Piscataway, New Jersey, USA).

Histopathology and immunohistochemistry

Histological assessment was performed on all tissue specimens. Morphological changes were evaluated in a blinded manner in each microscopic field, and the results of the

Table 1 Oligonucleotide primer sequences and polymerase chain reaction (PCR) conditions

mRNA		Sense and antisense primers	Expected size (bp)	PCR conditions (cycles)
MCP-1	Sense	5'-ACTATGCAGGTCTGTGTCACG-3'	417	94°C, 1'; 59°C, 1'; 72°C, 1' (30)
	Antisense	5'-AAGTGCTTGAGGTGGTGTGG-3'		
TGF- β	Sense	5'-CTTCAGTCCACAGAGAAGAACTGC-3'	289	91°C, 1'; 56°C, 1'; 72°C, 2' (32)
	Antisense	5'-CACGATCATGTTGGACAACCTGCTCC-3'		
PDGF-BB	Sense	5'-CAAGCCAGTCTCAAGAAGGCCAC-3'	148	94°C, 30'; 65°C, 30'; 72°C, 1' (34)
	Antisense	5'-AACGGTCAACCCGAGTTGAGGTGT-3'		
IL-1 β	Sense	5'-GTGTGATGTTCCCATAGACAGC-3'	383	94°C, 1'; 59°C, 1'; 72°C, 1' (33)
	Antisense	5'-TGAGAGGTGCTGATGACCAAGT-3'		
IL-6	Sense	5'-CCTTCCCTACTCACAAGTC-3'	501	94°C, 1'; 56°C, 1'; 72°C, 1' (34)
	Antisense	5'-GGATGGTCTTGGTCTTAGC-3'		
mMCP-1	Sense	5'-ATGAAAGTCTGCGCCCTT-3'	281	94°C, 1'; 60°C, 1'; 72°C, 1' (35)
	Antisense	5'-TCAAGTCTCGGAGTTGGGT-3'		
GAPDH	Sense	5'-TCCATGACAACCTTGGCATCTGG-3'	377	94°C, 30'; 60°C, 1'; 72°C, 1' (30)
	Antisense	5'-GTTGCTGTTAAGTCACAGGAGAC-3'		

MCP-1, monocyte chemoattractant protein 1; TGF- β , transforming growth factor β ; PDGF, platelet derived growth factor; IL-1 β , interleukin 1 β ; IL-6, interleukin 6; mMCP-1, mutant MCP-1; GAPDH, glyceraldehyde phosphate dehydrogenase.

Table 2 Time course of rat body weight (g) in each group

Group	Day 0	Day 4	Day 14	Day 28
DBTC+empty plasmid	203.6 (4.6)	174.9 (12.8)	183.4 (18.4)*	243.8 (24.8)**
DBTC+mMCP-1	201.2 (6.7)	178.2 (10.3)	217.0 (15.2)*	302.0 (14.4)**

DBTC, dibutyltin dichloride; mMCP-1, mutant monocyte chemoattractant protein 1.

Values are mean (SEM) (n=5).

Significant differences: *p<0.05, **p<0.01, DBTC+mMCP-1 treated group versus DBTC+empty plasmid treated group.

macroscopic findings were in accordance with Takano and colleagues.¹⁹

For immunohistochemistry, representative tissue sections were subjected to immunostaining for macrophages/monocytes using a primary monoclonal mouse anti-rat ED1 IgG antibody (Serotec; Dainippon, Osaka, Japan)²⁰ diluted to 1:200, and a development method (Nichirei, Tyokyo, Japan) was used for ED-1 positive cell detection. Immunostaining for MCP-1 using goat antirat MCP-1 IgG antibody (Santa Cruz, California, USA)²¹ and for α -SMA using mouse

monoclonal anti- α -SMA antibody (Dako, Kyoto, Japan), diluted to 1:100, was carried out and signals were then developed using an avidin-biotinylated peroxidase complex method. Morphometric analysis was performed with a microscope that had a computerised digital image analysis system (Mac scope) by two observers who were blinded to the treatment protocol. The number of ED1 positive cells were counted by two blinded observers in each section (five high power fields) and the average of the positive cells per section was reported.

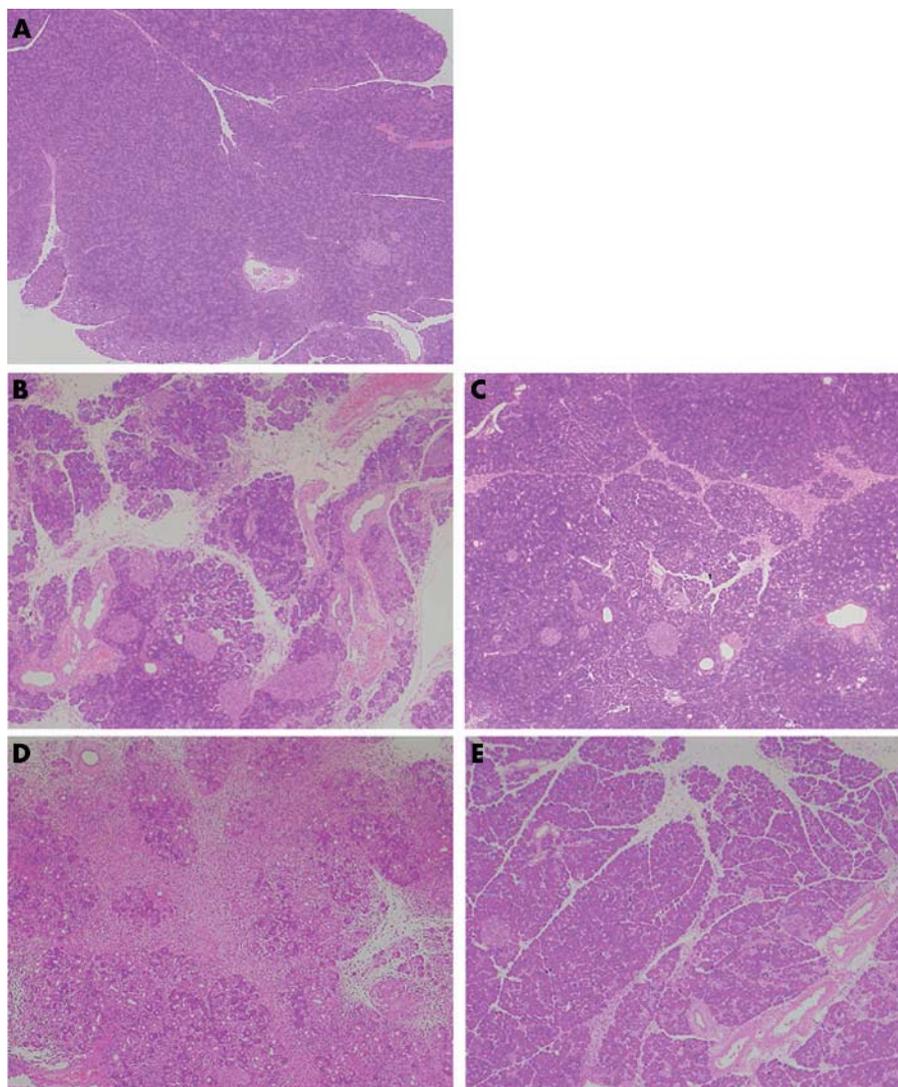


Figure 2 Microscopic findings in the pancreas. Rats transfected with the mutant monocyte chemoattractant protein 1 (mMCP-1) gene attenuated the development of pancreatic fibrosis induced by dibutyltin dichloride (DBTC). Representative photomicrographs of pancreatic tissue sections are shown. (A) Normal control rat. (B) DBTC+empty plasmid group on day 14. (C) DBTC+mMCP-1 group on day 14. (D) DBTC+empty plasmid group on day 28. (E) DBTC+mMCP-1 group on day 28. Haematoxylin-eosin stain; original magnification $\times 100$.

Table 3 Microscopic findings in the pancreas

Group	Oedema	Inflammatory cell infiltration	Fibrosis	Fatty change	Tubular complex
DBTC+empty plasmid (14 days)	0.6 (0.2)	2.3 (0.3)	2.1 (0.2)	1.4 (0.2)	2.0 (1.6)
DBTC+mMCP-1 (14 days)	0.3 (0.3)	1.7 (0.2)	1.6 (0.1)	1.1 (0.2)	1.5 (0.2)
DBTC+empty plasmid (28 days)	0	2.8 (0.2)**	2.7 (0.2)**	1.6 (0.1)**	2.8 (0.1)**
DBTC+mMCP-1 (28 days)	0	1.3 (0.1)**	1.3 (0.1)**	0.6 (0.1)**	1.0 (0.2)**

Histological findings of chronic pancreatitis such as fibrosis, inflammatory cell infiltration, tubular complexes, and fatty changes were calculated as the sum of lesion size scores per rat. Lesion size scores were as follows: 0 = absent; 1 = lesion appearing slightly in the lobule or intralobular region; 3 = lesion evident across the lobule and intralobular region or showing destruction of lobular architecture.

DBTC, dibutyltin dichloride; mMCP-1, mutant monocyte chemoattractant protein 1.

Values are means (SEM) (n = 5).

Significant differences: **p < 0.01, mMCP-1 treated group versus DBTC treated group on days 14 and 28.

Statistical analysis

Results are expressed as means (SEM). The Student's *t* test was used for statistical analysis. *P* values of <0.05 and <0.01 were accepted as statistically significant.

RESULTS

Histological evaluation of the pancreas after intravenous administration of DBTC

Following DBTC administration, the body weight of rats was decreased, probably because of pancreatic dysfunction. However, the mMCP-1 treated group preserved or prevented loss of body weight (table 2). In our study, pancreatic interstitial oedema and inflammatory cell infiltration, and pancreatic fibrosis were evident in the pancreas on day 14 (fig 2B) compared with normal rat pancreas (fig 2A). On day 28, we observed more widespread progression of interstitial fibrosis with infiltrating mononuclear cells, tubular complex formation, fatty change, and destruction of acinar cell and lobular architecture (fig 2D). In contrast, the mMCP-1 treated group inhibited early pancreatic inflammation on day 14 (fig 2C) and extended pancreatic fibrosis on day 28 (fig 2E) compared with the empty plasmid treated group. These microscopic results are described in table 3.

Effect of mMCP-1 on pancreatic content of protein and amylase, and serum concentration of MCP-1

Pancreatic protein in the empty plasmid treated group decreased significantly to 116.8 (13.9) mg/g on day 14 and to 99.1 (10.6) mg/g on day 28 compared with that of normal

rats (204.1 (11.9) mg/g) whereas in the mMCP-1 treated group it increased to 158.9 (11.4) mg/g on day 14 and to 176.6 (8.8) mg/g on day 28 (fig 3A). Similarly, pancreatic amylase in the empty plasmid treated group decreased significantly to 5693.0 (523.5) SU/dl on day 14 and to 4644.2 (154.3) SU/dl on day 28 compared with that of normal rats (28 369.6 (2024.1) mg/g) whereas in the mMCP-1 treated group it increased to 15 323.8 (2954.3) SU/dl on day 14 and to 23 311.3 (2065.8) SU/dl on day 28 (fig 3B).

Serum MCP-1 concentrations in the mMCP-1 treated group on days 14 and 28 (0.195 (0.024) ng/ml, 0.155 (0.005) ng/ml, respectively) markedly decreased compared with those in the empty plasmid treated group on days 14 and 28 (0.387 (0.051) ng/ml, 0.225 (0.023) ng/ml, respectively). However, MCP-1 levels were also low in normal rats (0.148 (0.002) ng/ml) (fig 3C).

mMCP-1 gene transfer prevented fibrosis and activation of pancreatic stellate cells (PSCs) in pancreatic tissue

Azan staining was associated with pancreatic fibrosis, which stained blue in Azan stained sections. The mMCP-1 treated group (fig 4B) showed less fibrosis than the empty plasmid treated group (fig 4A) on day 28. α -SMA was characterised as a marker of pancreatic stellate cell activation. The number of α -SMA positive cells completely resolved in mMCP-1 treated rats (fig 4D) compared with the empty plasmid treated rats (fig 4C) on day 28.

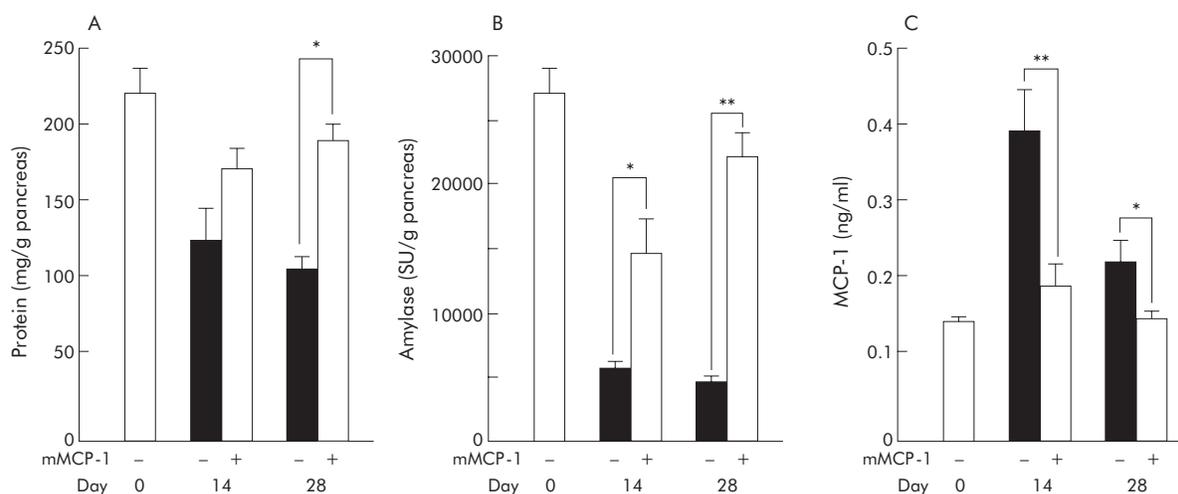


Figure 3 Effect of intramuscular transfer of the mutant monocyte chemoattractant protein 1 (mMCP-1) gene on pancreatic content of protein (A) and amylase (B), as well as serum MCP-1 concentrations (C) on days 14 and 28 after dibutyltin dichloride (DBTC) injury. Values are means (SEM) (n = 5). Significant differences: *p < 0.05, **p < 0.01, mMCP-1 treated group versus empty plasmid treated group.

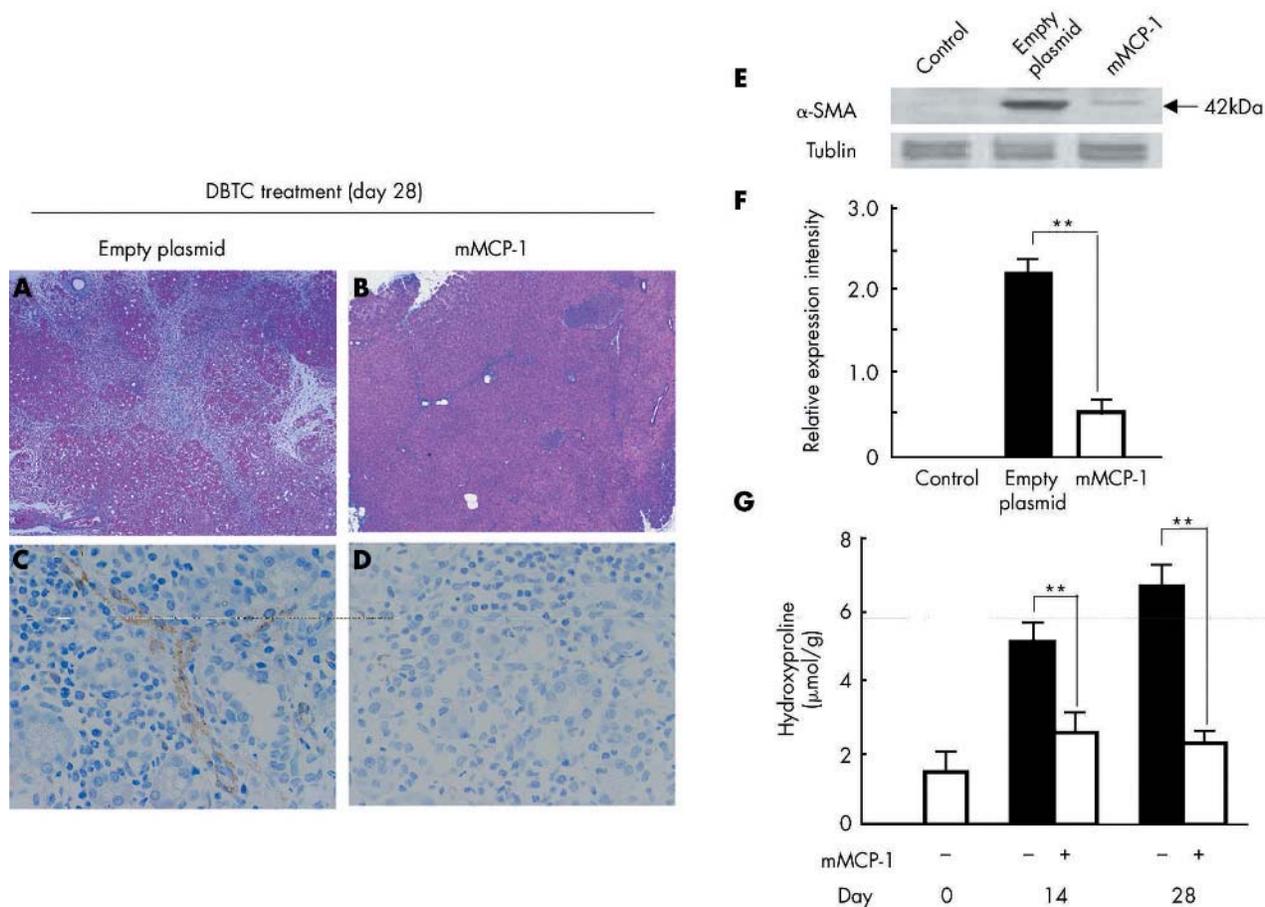


Figure 4 Administration of mutant monocyte chemoattractant protein 1 (mMCP-1) attenuated the severity indicators of pancreatic fibrosis. Pancreatic tissue sections from empty plasmid and mMCP-1 groups on day 28 after injury stained with Azan stain (A, B) and stained immunohistochemically for α smooth muscle actin (α -SMA) (C, D). (E) Representative western blot of α -SMA; tubulin was used as a loading control. (F) Densitometric analysis of mMCP-1 on pancreatic α -SMA protein expression on day 28 after injury. Grouped data are presented of four independent experiments. (G) Effect of intramuscular transfer of the mMCP-1 gene on pancreatic content of hydroxyproline on days 14 and 28 after dibutyltin dichloride injury. Values are means (SEM) (n=5). Significant differences: **p<0.01, mMCP-1 treated group versus empty plasmid treated group.

As shown in fig 4E, α -SMA protein expression was determined by western blot analysis, which detected an immunoreactive band with a molecular mass of 42 kDa. Pancreatic α -SMA expression of the empty plasmid treated group markedly increased on day 28 compared with normal rats whereas its expression was reduced by mMCP-1 gene transfer (fig 4F). Similarly, pancreatic content of hydroxyproline as a marker of fibrosis did not increase on days 1, 3, or 7 (data not shown) but significantly increased to 5.08 (0.51) $\mu\text{mol/g}$ on day 14 and to 6.66 (0.87) $\mu\text{mol/g}$ on day 28 compared with the control group (1.46 (0.23) $\mu\text{mol/g}$) whereas in the mMCP-1 treated group it was reduced to 2.56 (0.43) $\mu\text{mol/g}$ on day 14 and to 2.28 (0.26) $\mu\text{mol/g}$ on day 28 (fig 4G).

DBTC induced infiltration of monocyte/macrophage and MCP-1 expression were suppressed by mMCP-1 gene transfer

In rats, inflammation (ED-1 positive monocytes/macrophages) was markedly less in the mMCP-1 treated group (fig 5B) than in the empty plasmid treated group (fig 5A) on day 14, and especially on day 28. mMCP-1 treated rats (fig 5D) also had markedly reduced MCP-1 expression compared with the empty plasmid treated rats (fig 5C) on day 28. Monocyte/macrophage recruitment was prevented by mMCP-1 gene transfer (fig 5E).

mMCP-1 gene transfer reduced pancreatic cytokine and chemokine mRNA expression

Intrapancreatic mRNA expression of MCP-1, TGF- β , PDGF, IL-1 β , and IL-6 are shown in (fig 6A). The level of MCP-1 mRNA was almost undetectable in normal rats whereas on days 14 and 28 markedly increased MCP-1 mRNA expression was observed. In contrast, expression was limited by mMCP-1 gene transfer. Similarly, mRNA expression of other chemokines and cytokines was observed on days 14 and 28 after DBTC treatment and cytokines compared with the control group. mRNA expression was partly but significantly reduced in the mMCP-1 treated group (fig 6B).

Transgene expression via intramuscular injection

To measure mMCP-1 released by transfected skeletal muscle, mMCP-1 or empty plasmid at a dose of 50 $\mu\text{g}/\text{body}$ was administered into rat thigh muscles on day 4 after DBTC application. Regarding the level of transgene expression, we detected higher transgene expression in muscle than in serum (table 4).

DISCUSSION

Chronic pancreatitis is recognised as a chronic inflammatory disease, and is characterised by progressive loss of parenchyma and subsequent loss of pancreatic exocrine and endocrine function. Morphological findings in chronic

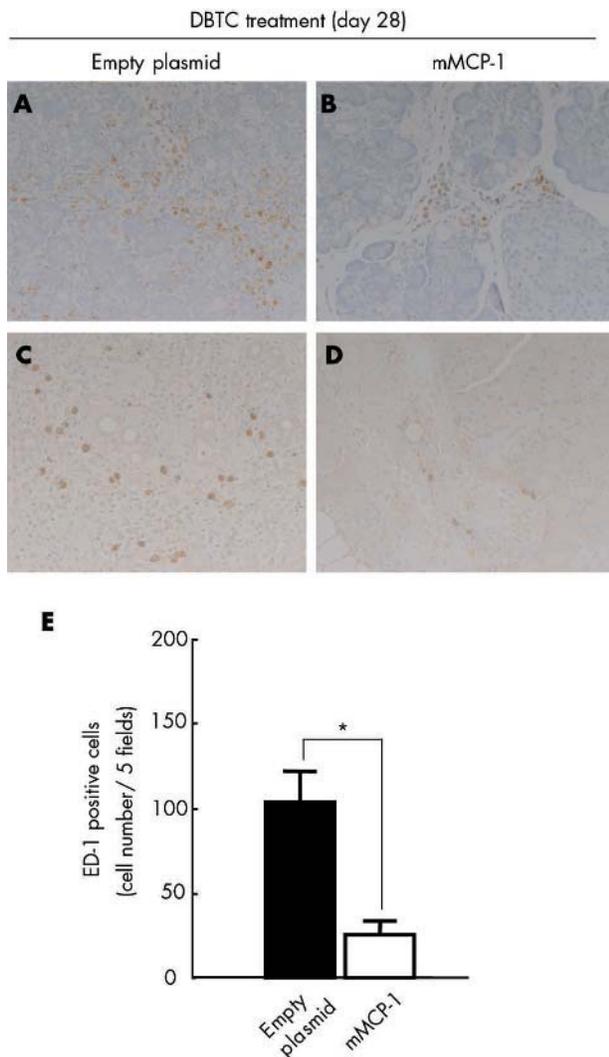


Figure 5 Immunohistochemistry of the pancreas for ED-1 (A, B), monocyte chemoattractant protein 1 (MCP-1) (C, D), and quantification of ED-1 positive cell number (E). Representative pancreatic tissue sections on day 28 after dibutyltin dichloride (DBTC) administration from a rat transfected with empty plasmid and a rat transfected with mMCP-1 gene were stained immunohistochemically for MCP-1 or ED1. Large mononuclear cells in the pancreatic interstitium were stained with anti-MCP-1 antibody (original magnification $\times 200$). ED-1 positive cells (brown labelled cytoplasm) indicating macrophages/monocytes, infiltrated into the pancreatic parenchyma (original magnification $\times 200$) in the pancreas. Total number was expressed. Values are means (SEM) ($n=4-5$). Significant differences: $*p<0.01$, mMCP-1 treated group versus empty plasmid treated group on day 28 after DBTC treatment.

pancreatitis, such as inflammatory infiltration of pancreatic tissue, fibrosis, atrophy of acinar cells, calcification, pancreatic duct strictures, etc., can be found at the very beginning of the disease. The second stage of the disease is characterised by various degrees of exocrine dysfunction, and later on endocrine dysfunction.²² Recent studies have suggested that recruitment of monocytes/macrophages play an important role in the development of chronic pancreatitis.⁷ Monocyte/macrophage recruitment to the inflammatory process releases various cytokines, growth factors, and chemokines. Chemokines induce cell migration and activation by binding to specific cell surface receptors on target cells. MCP-1 in particular may play a central role at the site of inflammation, resulting in initiation and progression of various diseases.

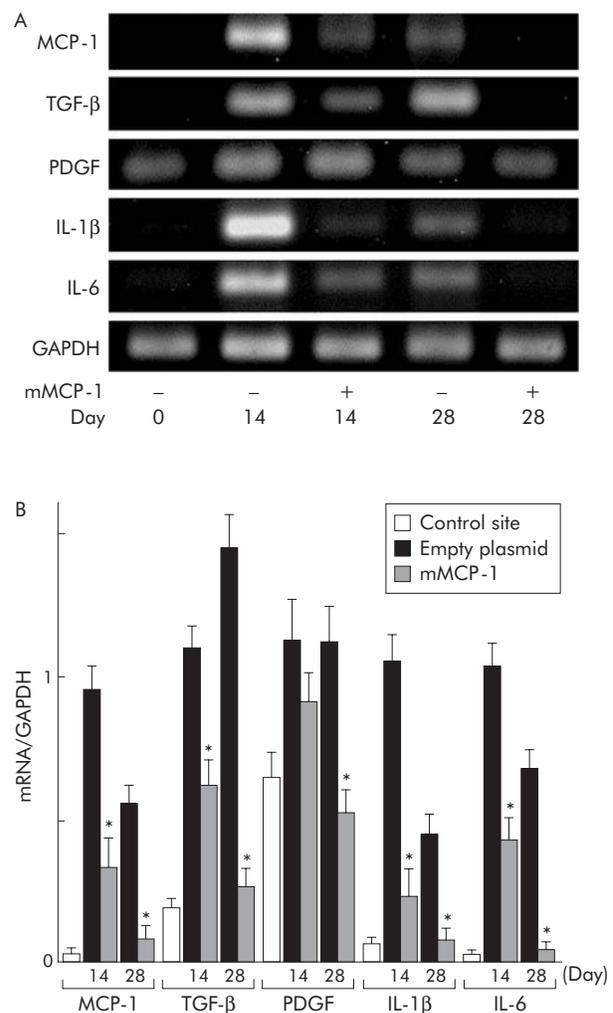


Figure 6 Expression of various inflammatory cytokines, chemokines, and growth factor. Each rat pancreas was harvested and frozen on the indicated days, and total RNA from pooled samples was isolated and subjected to reverse transcription-polymerase chain reaction (RT-PCR). The cDNA obtained from the pancreas was amplified with monocyte chemoattractant protein 1 (MCP-1), transforming growth factor β (TGF- β), platelet derived growth factor (PDGF), interleukin (IL-1 β), IL-6, and glyceraldehyde phosphate dehydrogenase (GAPDH). (A) Representative RT-PCR results for each group. (B) Relative intensity of PCR production bands were analysed using an NIH image, as described previously. Data are expressed as the ratio of each mRNA to the corresponding GAPDH mRNA. Values are means (SEM) ($n=3$). $*p<0.05$ mMCP-1 treated group versus empty plasmid treated group.

Rollins reported that mMCP-1 and normal MCP-1 formed a heterodimer, bound to CCR-2, and completely inhibited MCP-1 mediated monocyte chemotaxis in vitro.²³ Therefore, we performed the present study to evaluate the effect of mMCP-1 in blocking MCP-1 activity in vivo and determined whether it was effective in preventing the development of pancreatic fibrosis induced by DBTC in a rat model.

We demonstrated marked transgene expression of the mMCP-1 gene in the femoral region (the site of injection) by northern blot analysis, and mMCP-1 protein in serum was detected by western blot analysis.¹³ In our study, transgene expression was low in serum although its expression was highly detected in thigh muscle (table 4). One possible explanation is that the biological effect of mMCP-1 may be independent of plasma concentration. C-C chemokines, including MCP-1, are stored locally via binding to glycosaminoglycans in vivo,² suggesting that lower transgene

Table 4 Expression of the monocyte chemoattractant protein 1

Group	Muscle (human MCP-1) (pg/g)	Muscle (rat MCP-1) (pg/g)	Plasma(human MCP-1) (pg/ml)
Empty plasmid (day 3)	<31.2	411.1 (22.3)	<31.2
mMCP-1 (day 3)	4480.8 (591.3)**	337.9 (32.3)	<31.2

We assessed protein expression levels of mMCP-1 three days after gene transfer in plasma and muscle of rats using an MCP-1 ELISA kit. Values are means (SEM) (n=5~6). Significant difference: **p<0.01, mMCP-1 treated group versus the empty plasmid treated group.

expression of mMCP-1 may be sufficient to prevent MCP-1 activity. This is supported by previous data indicating that intramuscular plasmid mediated mMCP-1 injection inhibited over 80% of recombinant MCP-1 activity in the dermis *in vivo*.^{7,13}

In our study, pancreatic content of amylase and protein was improved by mMCP-1 gene transfer. For pancreatic fibrosis in particular, enzymatically, the pancreatic content of hydroxyproline, as an indicator of pancreatic fibrosis, was reduced by mMCP-1 gene transfer, and histologically, Azan stain showed less pancreatic fibrosis in the mMCP-1 treated group. Hence these findings indicate that pancreatic fibrosis induced by DBTC may be attenuated by mMCP-1 gene transfer. Immunohistochemically, suppression of MCP-1 producing cells and ED-1 positive cell infiltration into the pancreas were evident in the mMCP-1 treated group. Also, endogenous expression of MCP-1 in serum markedly

decreased after mMCP-1 gene transfer. Hence the implication is that the beneficial effects of mMCP-1 gene transfer may be caused mainly by suppression of monocyte and macrophage recruitment and activation. Therefore, early inhibition of the MCP-1 signal may prevent the autocrine/paracrine recruitment of monocytes/macrophages in the development of pancreatic fibrosis. Taken together, our data suggest that DBTC induced chronic pancreatitis was attenuated by mMCP-1 gene transfer, histologically and enzymatically.

It is reported that local and systemic production of many types of cytokines and chemokines are involved in the course of chronic pancreatitis as recruitment and activation of infiltrating cells are known to depend greatly on the local cytokine and chemokine production of inflammatory tissues.⁷ mMCP-1 gene transfer inhibited MCP-1, PDGF, and inflammatory cytokine (IL-1 β , IL-6) expression. These findings indicate that MCP-1 mediated inflammation may be

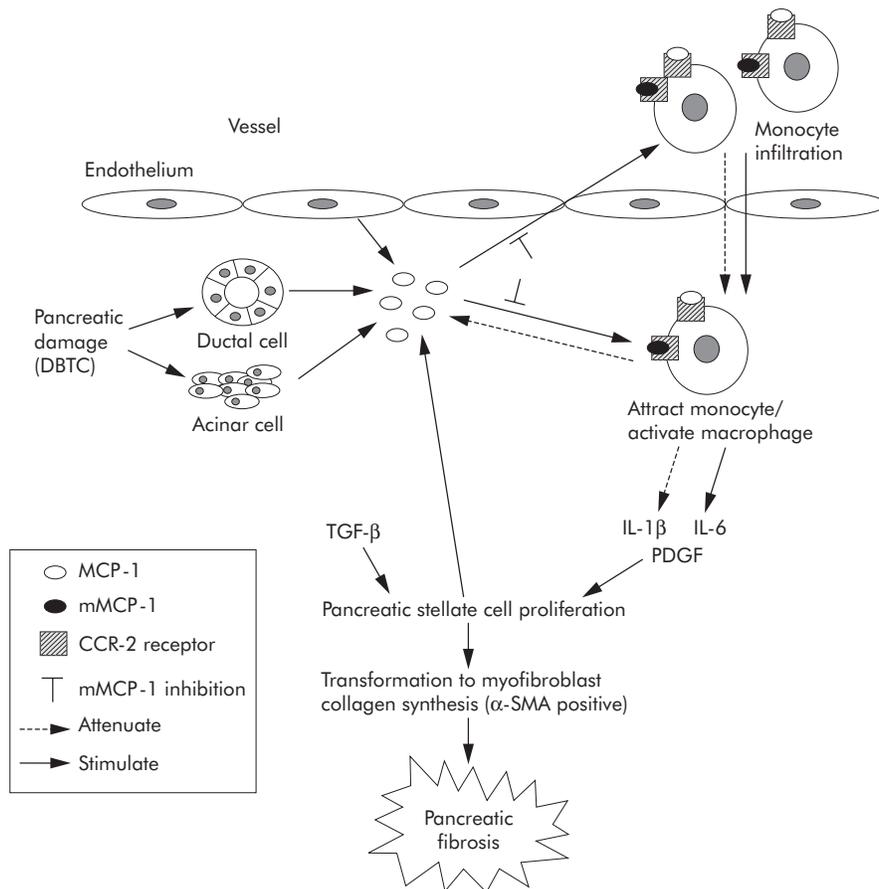


Figure 7 Hypothesis describing attenuation of the development of pancreatic fibrosis induced by dibutyltin dichloride (DBTC) in rats by the human dominant negative inhibitor of monocyte chemoattractant protein 1 (mMCP-1). CCR-2, C-C chemokine receptor; α -SMA, α smooth muscle actin; TGF- β , transforming growth factor β ; PDGF, platelet derived growth factor; IL-1 β , interleukin 1 β ; IL-6, interleukin 6.

attenuated by mMCP-1 treatment. IL-1 β is known as a proinflammatory cytokine and induces MCP-1 expression in activated PSCs.²⁶ Investigations concerning IL-6 indicate that it is an excellent predictor of disease severity and mediates the acute phase response.²⁴ PDGF plays a chemotactic effect on activated PSCs and increases PSCs migration.²⁷ Our results suggest that locally produced MCP-1 may not only affect recruitment of monocytes but also attract monocytes and activate macrophages to produce the inflammatory cytokines, chemokines, and growth factors in the development of pancreatic fibrosis.

Recently, in relation to pancreatic fibrosis, PSCs were identified and found surrounding blood vessels, pancreatic acini, and ductules.²⁵ Activation of stellate cells was accompanied by expression of the cytoskeletal protein α -SMA and produced most of the components of collagen and the extracellular matrix in vitro.²⁶ In our study, lesions in mMCP-1 transfer rats showed a decrease in α -SMA protein expression compared with empty plasmid treated rats. As shown in fig 7, MCP-1 was induced not only from monocytes/macrophages but also from injured acinar cells and ductal cells and probably also from other damaged pancreas tissue such as endothelial cells and smooth muscle cells. It is reported that activated pancreatic stellate cells produce MCP-1 following administration of lipopolysaccharide in a dose dependent manner.²⁸ More direct evidence for the involvement of MCP-1 in extracellular matrix deposition has been reported.⁷ Recent studies have suggested that MCP-1 acts as a fibrosis promoting chemokine; MCP-1 stimulated collagen gene expression via endogenous upregulation of TGF- β in rat lung fibroblasts.²⁹ TGF- β has a fibrogenic effect by stimulating synthesis of the extracellular matrix.³ MCP-1 may have a profibrogenic effect as clinically MCP-1 expression by myofibroblasts is increased in fibrous tissue sections from patients with chronic pancreatitis.⁷ Therefore, control of MCP-1 expression is thought to be an important therapeutic target for pancreatic fibrosis as well as inflammation.

In addition, timing of gene transfer needs to be considered. We observed that serum endogenous MCP-1 levels increased rapidly on day 1 after DBTC injection and then decreased on day 3. However, on day 7, levels increased again and slowly declined on days 14 and 28. Pancreatic content of MCP-1 showed peak levels one week later.¹⁰ Moreover, transgene expression via the intramuscular route showed peak production 3–4 days after injection and continued for at least 2–3 weeks.¹³ In this study, the mMCP-1 gene was injected at the times mentioned above. Using this gene transfer protocol may reduce early pancreatic inflammation and thus attenuate the development of pancreatic fibrosis.

Although this is the first report of anti-MCP-1 gene therapy for experimental chronic pancreatitis induced by DBTC in vivo, it has been reported that this strategy can be applied to other chronic inflammatory diseases, including atherosclerosis and restenosis,³⁰ pulmonary fibrosis,³¹ renal fibrosis,³² and liver fibrosis.^{33, 34}

In conclusion, our findings suggest that monocyte/macrophage recruitment and the systemic MCP-1 signal pathway contribute to the progression of chronic pancreatitis, and that blockade of MCP-1 may suppress the development of pancreatic fibrosis.

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Conflict of interest: None declared.

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EDITOR'S QUIZ: GI SNAPSHOT

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Answer

From question on page 1740

The axial computed tomography (CT) image (fig 1) shows peripheral fine branching intrahepatic air, consistent with portal venous gas. This was confirmed (arrow) on the coronal reconstructed CT image (fig 2), along with loops of dilated bowel. The coronal image (fig 3) showed reduced contrast enhancement near the origin of the superior mesenteric artery in keeping with occlusion (arrow).

Mesenteric arterial insufficiency has many causes but the abrupt onset in this case would be suggestive of embolic disease secondary to atrial fibrillation. In cases of mesenteric vascular disease, it is important to differentiate ischaemia from frank luminal infarction because the latter is associated with a dire prognosis. On plain radiographs the presence of portal venous gas is usually pathognomonic of transmural infarction. However, advances in CT technology allow

detection of tiny volumes of portal air and thus its presence is no longer considered necessarily premorbid. In fact, the presence of portal air on CT may be caused by non-ischaemic aetiologies in up to 20% of cases. The most reliable signs of transmural infarction in suspected mesenteric ischaemia are free air or fluid. The commonest CT sign in mesenteric ischaemia is bowel wall thickening, although absent in this case.

Early surgical intervention is essential in treating mesenteric ischaemia. However, as this case illustrates, portal venous gas when identified on the latest CT machines is not necessarily immediately premorbid, as this patient survived on conservative treatment for over 72 hours. It is also important to realise that when portal gas is identified on these CT systems, it is not pathognomonic for mesenteric arterial insufficiency and, as is usually the case in medicine, one must treat the patient and not the diagnostic test.

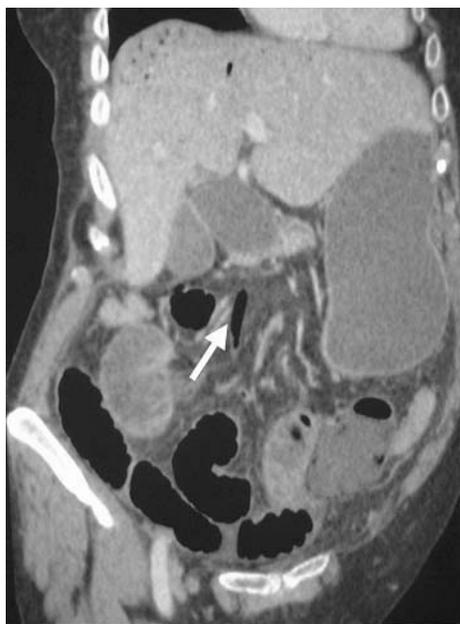


Figure 2 Coronal reconstructed computed tomography image showing portal venous gas, along with loops of dilated bowel.

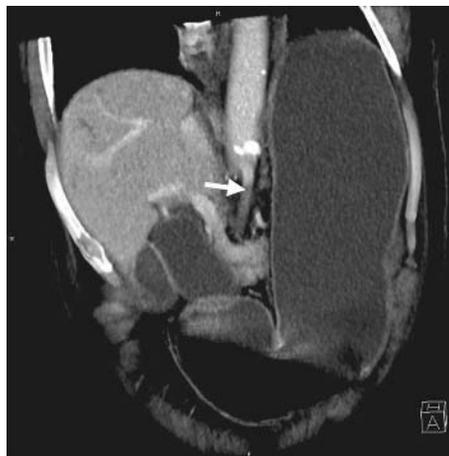


Figure 3 Coronal computed tomography image showing reduced contrast enhancement near the origin of the superior mesenteric artery in keeping with occlusion (arrow).

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