SYSTEMATIC REVIEW

Reappraisal of xenobiotic-induced, oxidative stress-mediated cellular injury in chronic pancreatitis: A systematic review

Ajith K Siriwardena

Ajith K Siriwardena, Hepatobiliary Surgery Unit, Manchester Royal Infirmary, Manchester M13 9WL, United Kingdom
Author contributions: Siriwardena AK solely contributed to this paper.
Correspondence to: Ajith K Siriwardena, MD, FRCS, Professor, Hepatobiliary Surgery Unit, Manchester Royal Infirmary, Oxford Road, Manchester M13 9WL,
United Kingdom. ajith.siriwardena@cmft.nhs.uk
Telephone: +44-161-2768886 Fax: +44-161-2764530
Received: September 19, 2013 Revised: November 5, 2013
Accepted: December 3, 2013
Published online: March 21, 2014

Abstract

AIM: To reappraise the hypothesis of xenobiotic-induced, cytochrome P450-mediated, micronutrient-deficient oxidative injury in chronic pancreatitis.

METHODS: Individual searches of the Medline and Embase databases were conducted for each component of the theory of oxidative-stress mediated cellular injury for the period from 1st January 1990 to 31st December 2012 using appropriate medical subject headings. Boolean operators were used. The individual components were drawn from a recent update on theory of oxidative stress-mediated cellular injury in chronic pancreatitis.

RESULTS: In relation to the association between exposure to volatile hydrocarbons and chronic pancreatitis the studies fail to adequately control for alcohol intake. Cytochrome P450 (CYP) induction occurs as a diffuse hepatic and extra-hepatic response to xenobiotic exposure rather than an acinar cell-specific process. GSH depletion is not consistently confirmed. There is good evidence of superoxide dismutase depletion in acute phases of injury but less to support a chronic intra-acinar depletion. Although the liver is the principal site of CYP induction there is no evidence to suggest that oxidative by-products are carried in bile and reflux into the pancreatic duct to cause injury.

CONCLUSION: Pancreatic acinar cell injury due to short-lived oxygen free radicals (generated by injury mediated by prematurely activated intra-acinar trypsin) is an important mechanism of cell damage in chronic pancreatitis. However, in contemporary paradigms of chronic pancreatitis this should be seen as one of a series of cell-injury mechanisms rather than a sole mediator.

Key words: Chronic pancreatitis; Cellular injury; Oxidative stress; Xenobiotics; Cytochrome P450

Core tip: This review systematically appraises the evidence for the key components of the hypothesis of environmental xenobiotic exposure leading to induction of the cytochrome P450 (CYP) system in the pancreatic acinar cell and in turn compromising the free radical quenching pathway in chronic pancreatitis. The central components of the hypothesis of xenobiotic-induced, micronutrient-deficient oxidative-stress mediated cell injury in chronic pancreatitis: xenobiotic exposure causing CYP induction, acinar CYP induction and hepatocyte mediated CYP by-products causing acinar injury by bile reflux do not withstand objective scrutiny.

INTRODUCTION

The oxidative stress hypothesis proposed that cellular injury in chronic pancreatitis (CP) originated at the level of the acinar cell and was principally mediated by short-lived oxygen-derived free radicals[1]. The hypothesis stated that these oxygen-derived free radicals were produced as a result of an imbalance between the processes producing these agents and those involved in de-activating or quenching them[1]. The endogenous peptide glutathione-{(2S)-2-amino-4-{[(1R)-1-[(carboxymethyl) carbamoyl]-2-sulfanylethyl] carbamoyl} butanoic acid} (GSH) is a central mediator in this model[2]. GSH is synthesized in the cytosol of all mammalian cells in a highly regulated manner[3]. The major determinants of GSH synthesis are the availability of cysteine and the activity of the rate-limiting enzyme, glutamate cysteine ligase (GCL)[3]. The sulfhydryl (thiol) group (SH) of cysteine serves as a proton donor and is responsible for the biological activity of glutathione[3].

The model for the aetiology and pathogenesis of chronic pancreatitis proposed by Braganza is multi-faceted and proposes that chronic exposure to xenobiotics, either through dietary imbalance, ingested toxins (such as alcohol) or inhaled toxins (such as volatile hydrocarbons) leads to chronic induction of both hepatic and pancreatic acinar cytochrome P450 (CYP)[1]. In the setting of chronic pancreatitis, CYP-mediated production of toxic metabolites then results in a disturbance of the equilibrium of endogenous oxidant quenching pathways and the resultant overwhelming of these pathways leads to unbalanced oxygen-free radical activity and acinar injury[4].

In their model, they propose that pancreatic acinar cells are susceptible to oxidant injury mediated by CYP-generated oxidative metabolites as there is a dearth of glutathione transferase in these cells, very little copper superoxide dismutase (required for free radical scavenging) and that the pool of cysteine is small[1].

Evidence in support of this model came from the demonstration that patients with chronic pancreatitis had increased serum/plasma levels of free radical oxidation products but lower levels of precursor methionine, thiols and glutathione[5,6]. Further, there was evidence that the dietary intake of some patients with CP was deficient in selenium, methionine and vitamin C, key cofactors in these transulfuration pathways[7].

A logical completion of this paradigm was the development of antioxidant therapy - a pharmacological preparation containing methionine, vitamin C, vitamin E and selenium designed to restore these critical co-factors to patients with CP[5,6]. Following clinical evaluation in Manchester UK, antioxidant therapy was introduced into regular clinical practice in that city initiating a polarisation of practice in pancreatology and a long-running debate which remains on-going, with the central question being whether antioxidant therapy is effective for pain control in chronic pancreatitis[8]. Proponents argued that positive clinical trials continued to accrue from outside Manchester[9-11]. Opponents argued that the evidence-base

remained small (Neoptolemos JP - personal communication). To address this problem, the Manchester group recently conducted and reported the largest randomized controlled trial of antioxidant therapy for treatment of pain in chronic pancreatitis - the ANTICIPATE study[12]. In this, 356 patients with CP were screened for eligibility, 92 randomised and 70 completed intervention with 6 mo of antioxidant therapy with antox version 1.2 (Pharmanord, Morpeth, United Kingdom) or matched placebo. Unique features of trial design were that the study focused exclusively on patients with radiological and clinical evidence of chronic pancreatitis but not solely on patients with end-stage disease. In keeping with modern trial design the study assessed treatment-related quality of life and both self-reported and clinician-assessed pain-related endpoints[13]. At the end of a 6 mo period of intervention with either drug or placebo there was no difference in the primary endpoint of abdominal pain assessed on an 11-point numerical rating scale with this finding being under-pinned by the absence of any difference in secondary endpoints of pain assessed by pain diaries or in quality of life assessed by validated questionnaires[12]. However, blood and plasma antioxidant levels were significantly elevated in patients in the antioxidant arm[12]. Although ANTICIPATE is the largest study of antox in chronic pancreatitis to date valid criticisms related to the inclusion of patients with prior pancreatic intervention and possibly also to the lack of screening for opiate addiction[14]. However, a striking finding is that the lack of benefit of antioxidant therapy seen in ANTICIPATE was also observed in patients with alcohol-related chronic pancreatitis in the large randomised trial from Delhi[15]. The Indian study used "reduction in painful days" as a primary endpoint and after 3 mo of intervention the mean difference between placebo and antioxidant groups was not significant for individuals with alcohol-induced chronic pancreatitis[15]. This clinical debate is likely to continue at least until the report of the currently on-going EUROPAC study evaluating antox in hereditary and idiopathic chronic pancreatitis[16].

However, these studies change the landscape of clinical evidence and the aim of the present study is to undertake a re-appraisal of the validity of the hypothesis of xenobiotic induced, CYP-mediated, micronutrient deficient oxidative injury in chronic pancreatitis, to integrate this hypothesis into current paradigms of the aetiology and pathogenesis of chronic pancreatitis and to provide direction for any future clinical evaluation of antioxidant therapy in this complex disease.

MATERIALS AND METHODS

Literature search strategy
The optimum structure for assessing the individual components of the oxidative stress hypothesis is to undertake a series of discrete searches. In order to do this, the components of the theory of oxidative-stress mediated cellular injury were isolated into the key elements proposed

by its authors. These components were drawn from a recent update on theory of oxidative stress-mediated cellular injury in chronic pancreatitis[1]: (1) Evidence that the inhalational route of xenobiotic entry allows for a "direct strike" to the pancreas; (2) Evidence for acinar cell induction of cytochrome P450 (CYP) in chronic pancreatitis; (3) Evidence that glutathione transferase is deficient in acinar cells in chronic pancreatitis; (4) Evidence that copper superoxide dismutase is deficient in acinar cells in chronic pancreatitis; (5) Evidence that CYP induction in hepatocytes leads to exposure of pancreatic acinar cells to oxidative stress by-products in chronic pancreatitis; and (6) Evidence that exogenous (dietary or medicinal) supplementation with antioxidants has a modulating effect on pancreatic injury in experimental chronic pancreatitis. It is accepted that there may be other components or facets of the theory but the features which are actively promoted as being central to the hypothesis are listed above.

For each individual component, a search of the Medline and Embase databases together with the web of knowledge[SM] was undertaken for the period from 1st January 1990 to 31st December 2012 using appropriate medical subject headings which are listed individually below. Boolean operators were used to ensure that variations in key words were captured in the searches. Searches were restricted to articles in the English language. Experimental evidence was included only in relation to the final category (evidence that exogenous supplementation with antioxidants modulates pancreatic injury). For all other categories, searches were restricted to clinical studies. Case reports, reviews, duplicate reports and papers not providing original data were not included. Abstracts of all papers identified in each search were reviewed and full papers of all potentially relevant articles were retrieved. Additional manuscripts were also identified from the bibliography of these reviewed papers. Data were extracted from the full paper by one individual. As the majority of the evidence is at the level of clinical case series, formal assessments for bias were not possible. Where there are potential sources of bias or distortion, these are individually highlighted. No pooling of data in the form of meta-analysis is undertaken in this study.

Individual component searches
Search 1: Inhalational xenobiotics and chronic pancreatitis: The search terms "inhalational xenobiotics", "xenobiotics", "chronic pancreatitis" and "pancreatitis" (All fields) yielded 26 articles. No additional articles were found after secondary searches using these words as topic headings in web of knowledge. After exclusion of reviews and articles relating to acute pancreatitis there were three studies which examined the association between xenobiotic exposure and chronic pancreatitis.

Search 2: Acinar cell induction of cytochrome P450 and chronic pancreatitis: The search terms "cytochrome P450" and chronic pancreatitis yielded 39 articles of which 34 were within the time limits of the search. The following were excluded: 9 reviews, 1 article on pan-

creatic cancer, 3 experimental studies, 4 on aspects unrelated to acinar cytochrome induction. No additional articles were found on secondary searches in web of knowledge using the topic terms "cytochrome P450" and "chronic pancreatitis".

Search 3: Glutathione transferase deficiency and chronic pancreatitis: The search terms "glutathione s-transferase" and "chronic pancreatitis" yielded 19 articles in PubMed and 26 when used as topic terms in web of knowledge (the additional articles in web of knowledge related either to liver disease or to pancreatic cancer).

Search 4: Copper superoxide dismutase deficiency and chronic pancreatitis: The search terms "super oxide dismutase" and "chronic pancreatitis" yielded 38 articles. Fourteen related to experimental studies, 3 to cancer and 2 to acute pancreatitis. Not all of the remaining 19 addressed the question of superoxide dismutase deficiency in chronic pancreatitis. When secondary searches were undertaken in web of knowledge no additional articles were found.

Search 5: CYP induction in hepatocytes and exposure of pancreatic acinar cells to oxidative stress by-products: The search terms "hepatocyte", "cytochrome P450" and "chronic pancreatitis" yielded 3 articles. No additional articles were found by secondary searches in web of knowledge. Only one of these 3 articles addressed the relationship between hepatocyte-derived agents and chronic pancreatitis.

Search 6: Antioxidant supplementation and modulation of pancreatic injury in experimental chronic pancreatitis: The terms "antioxidant" and "experimental chronic pancreatitis" were first assessed and yielded 34 articles in PubMed and 35 in web of knowledge. Examined in detail, the 34 citations in PubMed included 8 reviews, 8 studies on experimental acute pancreatitis, 5 studies on mechanism of disease in chronic pancreatitis, 4 clinical studies, 1 article in Russian and 1 on liver disease. The additional study identified in web of knowledge was retrieved and a total of 8 studies of various antioxidants or antioxidant-like interventions in experimental chronic pancreatitis were examined. Broader search criteria such as "animal models of chronic pancreatitis" yielded 332 articles including further intervention studies but it is not clear whether the interventions target oxidative injury and thus the more restrictive criteria were adopted for this study, accepting that not all relevant articles may have been captured.

Clinical trials of antioxidant supplementation in chronic pancreatitis have not been included in this re-appraisal a they have been summarised extensively elsewhere[1,5-7,9-11].

RESULTS
Evidence that the inhalational route of xenobiotic entry allows for a "direct strike" to the pancreas
Occupational exposure to hydrocarbons was proposed

as a risk factor for chronic pancreatitis with these compounds hypothesised as inducers of phase I CYP 450 in the pancreas and liver. McNamee undertook a case control study comparing likely hydrocarbon exposure in 102 patients with chronic pancreatitis to 204 age and gender-matched reference cases[17]. This remains to date, the only study of its kind. The reference cases were selected as they lived in the same localities as the index cases and thus could be hypothesised to have similar environmental hydrocarbon exposure (although occupational exposure would potentially be different). A potential methodological limitation of the study is that it uses a previously unreported and unvalidated "exposure to hydrocarbon" score which categorises exposure into discrete groups: "zero", "low" and "high".

The main findings were that 56 (55%) of cases and 81 (40%) of controls had a hydrocarbon exposure score of > zero. McNamee reported an OR for hydrocarbon exposure in cases with chronic pancreatitis compared to controls of 2.21 (90%CI: 1.38-3.53). It should be noted that 48 (47%) of the cases consumed alcohol to excess ($\geq$ 50 units per week for men, $\geq$ 35 units per week for women) compared to only 44 (22%) of the controls[17].

In a more modern study, Jeppe and Smith examined xenobiotic exposure in patients undergoing surgery for chronic pancreatitis in Johannesburg, South Africa[18]. Their findings are similar to those of McNamee. Although 86 (77%) of their 112 patients had been exposed to hydrocarbons in the form of burning firewood and coal, 104 (93%) consumed alcohol with 44 (39%) taking this daily[18].

Segal and colleagues demonstrated that alcohol intake was higher in 21 patients with alcoholic psychosis than 14 controls with chronic pancreatitis[19]. Patients with CP had higher scores for exposure to occupational xenobiotics than alcoholics. No causal relationship was demonstrated between hydrocarbon exposure and chronic pancreatitis.

Evidence for acinar cell induction of CYP in chronic pancreatitis

A mainstay of the original concept of oxidative stress-mediated injury was a recognition that the pancreas was one of the extrahepatic sites of production of cytochrome P450[1]. Chronic exposure to xenobiotics, either in the form of ingested toxins such as alcohol or inhaled volatile chemicals was hypothesised as leading to CYP induction. In turn, CYP metabolism led to the intra-acinar generation of toxic free radicals[1]. Braganza's group provided early evidence to substantiate this hypothesis in a study examining immunohistochemical patterns of expression of four phase I drug-metabolizing enzymes (cytochromes P-450 ⅢA1, P-450 ⅡE, P-450 Ⅰ A2, and NADPH cytochrome P-450 reductase) and one phase Ⅱ enzyme [glutathione S-transferase (GST) 5-5] in normal donor pancreata and pancreatic tissue from six patients with chronic pancreatitis and a further six with pancreatic cancer[20]. They categorised their results according to those cytochromes were there was more than 50% increase in immunostaining compared to normal

donors (on a 4 point scale from "no stain" to "intense"). With respect to pancreatic acini, this increase was seen in CYP-450 Ⅲ A1, CYP-450 IA2 and NADPH P-450 reductase (Table 1). This phenomenon was not specific to the chronic pancreatitis patients but was also seen in pancreatic cancer and was also seen in hepatocytes and pancreatic islets. There was no functional correlate of this immunostaining.

Wacke's study builds on these findings of cytochrome induction in specimens from patients with chronic pancreatitis by assessing the functional ability of microsomal fractions (containing 200 μg protein) from normal pancreata and chronic pancreatitis patients to undertake oxidative biotransformation of verapamil[21]. This study also shows increased immunostaining - both in terms of numbers of cells and also in terms of intensity for cytochrome P450 enzymes in tissues from chronic pancreatitis. There are differences between Foster's findings and those of Wacke which are attributed by the later authors to the different antibodies used as Foster's study used antibodies raised against rat liver whereas the later study used recombinant human antibodies. Wacke demonstrated increased verapamil biotransformation to norverapamil in microsomal fractions taken from chronic pancreatitis tissue[21]. Similar increases in pancreatic cytochrome P450 expression were shown in a separate study by Standop et al[22] although they showed more expression in islets than in pancreatic acinar cells.

Over the last 20 years, there have been important progressions in knowledge of cytochrome P450 enzymes. Humans have 57 genes divided amongst 18 families of cytochrome P450 genes[23]. Fifteen human CYPS (all from the CYP1, CYP2 and CYP3 families) are known to be involved in xenobiotic metabolism, with a central role in phas I drug metabolism[23]. In man, the principle members of the CYP1 family are CYP1A1, 1A2 and 1B1[23]. Constitutive expression is very low but is inducible by exposure to aromatic hydrocarbons. The CYP2 family contribute to xenobiotic metabolism of drugs and alcohol. Current knowledge indicates that CYP 2E1 is predominantly responsible for alcohol metabolism[24]. The CYP3 family constitutes 3 members in humans and is predominantly involved in drug metabolism[25]. Current knowledge also includes awareness of transcriptional regulation of cytochrome P450 function, functional polymorphisms at CYP gene loci, potentially relevant knowledge of the propensity for acinar cells to trans-differentiate under certain conditions such as chronic glucocorticoid exposure to assume hepatocyte characteristics and evidence of substantial inter-species variation in expression patterns of these enzymes[26-29]. Consistent findings include the dominance of islet expression of cytochromes over acinar expression and the independence of CYP 450 signalling from regulation by cytosolic calcium[29-31].

Evidence that glutathione transferase is deficient in acinar cells in chronic pancreatitis

GST family of enzymes are a diverse family of dimeric proteins found principally in the cytoplasm where they have a

Table 1 Evidence for cytochrome induction in pancreatic acinar cells in chronic pancreatitis

Ref.	Cytochrome	Method of assessment	Histologic verification	Increased expression in acinar cell	Functional correlate
Foster et al[20]	CYP 3A1 CYP 2E CYP1A2 NADPH 450[1] Glutathione	Immunohistochemical staining > 50% of healthy donor	No	CYP 3A1 CYP1A2 NADPH 450[1]	No
Wacke et al[21]	CYP1A1 CYP1A2 CYP2C9 CYP2E1 CYP3A1	No of cases having high ratios compared to control	Yes	CYP1A1[2] CYP1A2 CYP2E1 CYP 3A1[2]	Yes (verapamil biotransformation)
Standop et al[22]	CYP1A1, 2 2B6 2C 8/9/19 2D6 2.00E + 01 3A4 NA-OR	Differences in frequency of immunopositivity between normal, CP and cancer	Yes	CYP1A CYP 2B6 CYP 2C8/9/19 2D6 NA-OR	No

[1]NADPH cytochrome P 450 reductase; [2]Expression similarly increased in normal non-diseased controls. CYP: Cytochrome P450; NA-OR: NADPH oxio reductase.

central role in phase II xenobiotic metabolism[32]. The oxidative stress hypothesis proposed that in contrast to induction of CYPs in chronic pancreatitis there was a reduction in acinar GSTs thus leading to failure of detoxification[1].

Ulrich et al[33] studied immunohistochemical staining for glutathione S-transferase-π (pi) in normal pancreata and chronic pancreatitis. There was no immunostaining in acinar cells from either normal pancreata or chronic pancreatitis[33]. In contrast, there was increased staining in the islets of patients with chronic pancreatitis compared to normal. From this evidence, the authors concluded that "toxic substances have a role in the genesis of CP and suggest an important role of the islet cells in protecting the exocrine pancreas"[33]. The mechanisms whereby increased islet GST protects pancreatic acinar cells is unclear.

These authors then went on to examine whether the increased number of islet cells expressing GST-π and the absence in the acinar cells are compensated for by other GST iso-enzymes. They investigated the expression of GST-α and GST-μ in the same specimens and found no significant differences between normal and chronic pancreatitis.

Foster's original study, which examined immunohistochemical expression of glutathione S-transferase 5-5 showed that staining was preserved in chronic pancreatitis as compared to normal[20].

Integrating this information into an increasingly complex picture, it seems difficult to argue a case for GST deficiency when the findings simply demonstrate that there was no difference in expression pattern between normal and chronic pancreatitis.

Evidence that copper superoxide dismutase is deficient in acinar cells in chronic pancreatitis

Superoxide dismutase (SOD) are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide and are important antioxidant mechanisms[34].

There are three major families of superoxide dismutase, depending on the metal cofactor: copper/zinc (Cu/Zn) (which binds both copper and zinc), Iron (Fe) and manganese (Mn) types (which bind either iron or manganese), and the Ni type, which binds nickel[34].

Intra-acinar deficiency of SOD was the fourth pillar of the hypothesis of oxidative stress-mediated cellular injury[1]. The evidence for this was from Hausmann's study which showed that although levels of Cu/Zn SOD were elevated in the pancreatic juice of patients with chronic pancreatitis, acinar levels detected by immunostaining were very low[35]. Milnerowicz conducted similar studies more recently but measured zinc, copper, metallothionein and Cu/Zn superoxide dismutase activity[34]. Elevated serum Cu levels together with a significant increase of Cu/Zn superoxide dismutase activity was observed in the blood of patients with chronic pancreatitis and chronic pancreatitis. In slices of the pancreas during pancreatitis, they observed variable immunohistochemical staining for Cu/Zn SOD[34].

Current knowledge of the genetics of superoxide dismutase acknowledge genetic variations in expression leading to variation in function[36]. Although relating to acute pancreatitis, Abu-Hilal et al[37] demonstrated that the erythrocyte content of superoxide dismutase was reduced during the clinical course of acute pancreatitis[37]. In an experimental model, superoxide dismutase knockout (SOD -/-) resulted in mice that were more susceptible to cerulein-induced pancreatic injury[38]. Finally, in an elegant study (again in experimental acute pancreatitis) Cuzzocrea et al[39] demonstrated that intervention with a superoxide dismutase mimetic M40401, attenuated pancreatic and remote organ injury.

Evidence that CYP induction in hepatocytes leads to exposure of pancreatic acinar cells to oxidative stress by-products in chronic pancreatitis

Xenobiotic exposure at the hepatocyte level leads to in-

duction of phase I cytochrome P450 xenobiotic-metabolising enzymes. As the hepatocytes are quantitatively the predominant source of CYP 450 in man, Braganza proposed that their oxidant by-products may contribute to pancreatic injury by passage along the bile duct and reflux into the pancreatic duct. This theory led to the highly idiosyncratic operation of bile duct ligation to divert bile in patients with chronic pancreatitis[40]. Even 20 years after these operations, it is of concern that the report makes no mention of any institutional or regional ethics review prior to subjecting these patients to surgery. In the two patients reported in this index study, attacks of pancreatic pain continued after the surgery. There is little doubt that biliary diversion in man has no role to play in modulating episodes of pancreatic pain in chronic pancreatitis.

Evidence that exogenous (dietary or medicinal) supplementation with antioxidants has a modulating effect on pancreatic injury in experimental chronic pancreatitis

The difficulty in assessing the effect of antioxidant intervention in experimental chronic pancreatitis lies partly in the difficulty of reproducing this disease in animal models[41]. In particular, there has historically been a dearth of reproducible models of visceral pain in chronic pancreatitis[42]. These difficulties are reflected in the heterogeneity of animal models of chronic pancreatitis (Table 2).

Gómez *et al*[43] used cyclosporin A to transform the acute pancreatic injury produced by caerulein injection to a more chronic form characterised by acinar loss and fibrosis. Oxidative stress was present in this model as measured by increased levels of thiobarbituric acid-reactive substance and 8-isoprostanes after induction of pancreatitis. There was also increased fibrosis as measured by an increase in transforming growth factor-β (TGF-β). This combination of inflammation and fibrosis is regarded as a mimic for human chronic pancreatitis. Intervention with vitamin E reduced lipoperoxidation, TGF-β bioactivity and increased myofibroblast apoptosis leading to a reduction in pancreatic injury and fibrosis[43].

Yoo *et al*[44] reported similar improvements in a murine chronic pancreatitis model following intervention with DA-9601, a novel phytochemical agent. de Las Heras-Castaño *et al*[45] also used the rat caerulein/cyclosporine model and demonstrated that intervention with compound antioxidant therapy similar to the combinations used in clinical practice was associated with a reduction in pancreatic fibrosis and reduction in glutathione peroxidase but no difference in antioxidant status. Mas *et al*[46] reported that taurine reduced pancreatic fibrosis in a model of CP produced by intraductal infusion of TNBS and concluded that "Antioxidant treatment might be considered a novel option to alleviate the fibrotic process in CP". Other interventions associated with a favourable outcome in the treatment group compared to animals with experimental chronic pancreatitis include ascorbic acid[47], pravastatin[48], α-tocopherol[49,50] and taurine[51].

Difficulties in interpreting these studies are that the effects of many of the intervention agents are pleomorphic with antioxidant, anti-inflammatory and anti-fibrotic properties. Table 2 also illustrates the relatively short time course of these models and the difficulties inherent in extrapolating to the clinical setting. An overview would suggest that experimental antioxidant-type interventions are able to target pancreatic inflammatory pathways and modulate pancreatic injury.

DISCUSSION

This study undertakes a re-appraisal of the evidence for the hypothesis of xenobiotic-induced, micronutrient deficient, oxidative stress-mediated cellular injury in chronic pancreatitis. This reassessment is timely in light of the difference in findings between the ANTICIPATE study of antox in chronic pancreatitis and the earlier study from India. Although these differences have been carefully and critically examined[52], the underlying hypothesis has not been critically re-appraised since its introduction in the 1980s. Thus before any further studies of antioxidant therapy are undertaken it is necessary to re-assess the hypothesis and in particular to integrate this model into contemporary paradigms of chronic pancreatitis.

Two important areas of potential bias should be considered. First, it is accepted that the narrative structure of the review could be regarded as a potential limitation as manuscript inclusion and exclusion could be subject to unreported bias. However, this is style of review is necessary given the relatively limited volume of evidence and the fact that many of the key articles are over 30 years old. It is thought that all major evidence has been cited. A second potential bias that should be discussed revolves around the antagonistic interpretation often generated by this hypothesis. Specifically, would an article from the current Manchester group adopt a biased stance? It should be remembered that the current group conducted the ANTICIPATE study which is the largest intervention trial of antox-based antioxidant therapy in chronic pancreatitis. The group have credibility in design and execution of balanced clinical trials in this area[53] but equally need to undertake due diligence in appraising the evidence in a constructive fashion before embarking on further clinical trials[54].

Interpreting the results bearing these potential limitations in mind, the first element of the hypothesis to consider is that of a causal association between exposure to inhalational xenobiotics and chronic pancreatitis[1]. Several important facts emerge. First, it is clear that there are few studies to examine this question. Second, the types of volatile hydrocarbon (if any) associated with pancreatic injury are unclear. Third, the key finding is that the studies fail to adequately control for alcohol intake. For example, in the McNamee study 48 (47%) of the cases consumed alcohol to excess ($\geq$ 50 units for men, $\geq$ 35 units for women) compared to only 44 (22%) of the controls[17]. For sake of balance, it should be pointed out that in a review, Braganza *et al*[55] cited several instances

Table 2 Interventions with evidence of modulation of experimental chronic pancreatitis

First Author	Animal	Model of CP	Intervention	Modes of action	Time course and point of intervention	Pancreatic parenchymal end-points	Systemic end-points
Gómez et al[43]	Rat	ip cyclosporine A and ip caerulein	Vitamin E (gavage)	Anti-oxidant properties: control oxidative radicals Anti-oxidant independent properties: reduction of fibrosis	15 d study Intervention day 8	Reduced peroxidation (TBARS level) Reduced collagen and hyaluronic acid deposition Reduced acinar atrophy and periductal fibrosis	Reduced plasma lipoperoxidation. Reduced plasma TGF-β
Yoo et al[44]	Mouse	ip caerulein	DA 9601	Phytochemical possessing anti-inflammatory and antioxidative action	35 d Intervention weekly from week 1	Reduced pancreatic fibrosis Myeloperoxidase and iNOS activities decreased HSP-70 increased	None
de Las Heras-Castaño et al[45]	Rat	ip cyclosporin A and ip caerulein	L-methionine β-carotene ascorbic acid α-tocopherol selenium	Antioxidant	49 d Intervention weekly from week 1	Reduction in pancreatic fibrosis Similar cellular glutathione peroxidase levels to control and less than caerulein alone group Increased total antioxidant status but also increased in caerulein alone group	None
Mas et al[46]	Rat	Intraductal TNBS	Taurine	Anti-fibrotic	28 d Intervention weekly from week 1	Tissue malondialdehyde levels increased SOD and glutathione peroxidase activities decreased Histopathologic injury scores lower	Reduction in weight loss
Lu et al[47]	Rat	DBTC infusion[1]	Ascorbic acid	Antioxidant	56 d Intervention weekly from day 28	Increased SOD and reduce malondialdehyde No change in pancreatic fibrosis Less atrophy	Reduce serum hyaluronic acid
Wei et al[48]	Rat	PD infusion of pancreatic juice/bile (PD hypertension)	Pravastatin 10 mg/kg per day	Competitive inhibition of 3-hydroxy-3-methylglutarylcoenzyme A reductase	14 d Intervention from day 2	Maintained secretin-stimulated secretory response Attenuated fibrosis with less expression of TGF-β mRNA Maintained pancreatic exocrine function	Amelioration of weight loss
Li et al[49]	Rat	Intraductal TNBS	α-tocopherol	Antifibrotic	28 d Intervention daily	Reduced incidence of pseudocysts from 100% to 69% Ameliorates pancreatic fibrosis	Amelioration of weight loss
Jiang et al[50]	Rat	DBTC	α-tocopherol and tocotrienol-rich fraction	Antioxidant and antifibrotic	28 d Intervention daily (oral)	Reduced pancreatic inflammation and fibrosis Down-regulated mRNA expression of TGF-β1 and collagen-α1	None
Matsushita et al[51]	Rat	DBTC	Taurine	Antifibrotic	28 d Intervention day 0	Inhibition of pancreatic fibrosis Decreased apoptotic index	None

[1]Dibutylin dichloride (DBTC) given by infusion into tail vein. TBARS: Thiobarbituric acid-reactive substance; TGF-β: Transforming growth factor beta; iNOS: Inducible nitric oxide synthase; HSP: Heat shock protein; TNBS: Trinitrobenzene sulfonic acid; SOD: Superoxide dismutase; PD: Pancreatic duct.

of patients with pain related to exposure to occupational hydrocarbons whose symptoms settled on avoidance but returned on re-exposure. At the least, these findings require independent, large scale validation using a validated hydrocarbon exposure score and carefully controlling for other factors such as alcohol intake before acceptance. The available evidence does not support a conclusion that xenobiotic exposure by itself should continue to be

regarded as a risk factor for chronic pancreatitis.

The second element of the hypothesis is that of pancreatic cytochrome induction[1]. Integrating the findings into a modern framework there is good evidence that CYP induction occurs as a diffuse hepatic and extrahepatic response to xenobiotic exposure. However, there is little evidence to support the hypothesis of preferential acinar cell CYP induction. CYP expression in the pancre-

atic acinar cell is quantitatively small (although the resistance of acinar cells to phase I metabolites is unknown) and the contemporaneous evidence from the three immunohistochemistry studies of preferential expression relates principally to islets[20-22]. To take a balanced view the link between intra-pancreatic CYP induction and acinar injury should be regarded as an association with no evidence that the former is causatively linked to chronic pancreatitis.

The third element of the hypothesis relates to intra-acinar depletion of GSH[1]. The findings of the review show evidence of increased islet expression of GSH and that the original study by Foster *et al*[20] showed that immunostaining for GSH was preserved in chronic pancreatitis tissue. Integrating this evidence into modern concepts, it is likely that endogenous cell-defence pathways are compromised during the injury phases of chronic pancreatitis but there is little evidence to suggest that the GSH transulfuration pathway is uniquely important in this process.

The fourth element of the hypothesis is intra-acinar superoxide dismutase deficiency[1]. There is good evidence of SOD depletion in acute phases of injury but less to support a chronic intra-acinar depletion. Integrating current knowledge into the oxidative stress injury hypothesis would suggest that although superoxide dismutase biology is more complex than previously thought, intra-acinar deficiency of quenching activity may play a part in cellular injury in chronic pancreatitis.

The fifth element is that CYP induction in hepatocytes leads to exposure of pancreatic acinar cells to oxidative stress by-products by reflux of bile into the pancreatic duct[1]. Although the liver is the principal site of CYP induction there is no evidence to suggest that oxidative by-products are carried in bile and reflux into the pancreatic duct to cause injury.

Finally, in relation to experimental chronic pancreatitis, there is evidence of antioxidant modulation of pancreatic injury: tocotrienol supplementation reduced pancreatic inflammatory gene expression in an experimental rat model of chronic pancreatitis[56]. Other interventions associated with modulation of the disease course of experimental chronic pancreatitis are seen in Table 2. It is difficult and possibly inappropriate to attempt to synthesise a single common pathway of action for these agents. In acute pancreatitis, many interventions are effective in experimental models but none translate to a clinical setting and these limitations may also apply in relation to experimental chronic pancreatitis[57]. In particular, although these experimental studies suggest that exogenous supplementation with compounds with antioxidant properties may modify pancreatic injury, there is insufficient evidence to extrapolate these findings to the clinical setting[57].

To integrate these findings into modern paradigms of chronic pancreatitis, the epidemiology of chronic pancreatitis suggests a complex disease, predominantly associated with prolonged, heavy consumption of alcohol but also associated with several other causes[58,59]. Population-based registries have allowed for better definition of the

incidence of the disease and this process of identification has occurred in the latter half of the twentieth century[60,61]. To seek an association between this process of better definition of disease and hydrocarbon exposure in industrialised countries is tempting but the available literature does not support a causal association between xenobiotic exposure and pancreatic acinar injury in chronic pancreatitis.

The cationic trypsinogen codon 122 R122H mutation was the first mutation clearly associated with chronic (and acute) pancreatitis[62]. The cationic trypsinogen R122H mutation is a "gain of function" mutation as it prevents trypsin inactivation[63]. Early and inappropriate intra-acinar trypsin activation is now recognised as an important proximal pathway step in chronic pancreatitis[64]. Other mutations including those at the serin protease inhibitor Kazal type I /pancreatic secretory trypsin inhibitor I (SPINK1/PT1) mutation are also associated with chronic pancreatitis[65] and it is now understood that in genetic terms CP is a complex and heterogeneous disease.

Pancreatic injury in CP is characterised by fibrosis and the role of the extracellular matrix and pancreatic stellate cells in mediating this component of injury is now better recognised[66]. Similarly, alterations in the composition of pancreatic nerves, neurotransmitters and the concept of neural remodelling[67] together with alterations in central nervous system perceptions of pain[68] are now recognised to be important component of the perception of pain in this disease.

In conclusion, pancreatic acinar cell injury due to short-lived oxygen free radicals (generated by injury mediated by prematurely activated intra-acinar trypsin) is an important mechanism of cell damage in chronic pancreatitis. However, this should be seen as one of a series of cell-injury mechanisms rather than as a sole mediator. The central components of the hypothesis of xenobiotic-induced, micronutrient-deficient oxidative-stress mediated cell injury in chronic pancreatitis: xenobiotic exposure causing CYP induction, acinar CYP induction and hepatocyte mediated CYP by-products causing acinar injury by bile reflux do not withstand objective scrutiny. This view is essentially mirrored by the low use of antioxidant therapy in terms of non-analgesic medication for chronic pancreatitis[69]. In terms of the cost and ethical considerations around subjecting vulnerable patients to further trials, current clinical evidence would argue strongly against efficacy of exogenous antioxidant therapy in alcohol-related chronic pancreatitis. Although there may be options for evaluation in non-alcohol related disease the central tenets of the hypothesis are insufficiently strong even to justify this. Rationally, it is time to end the on-going debate about antioxidant therapy in chronic pancreatitis and to look for alternative truly effective interventions for patients with this dreadful disease.

COMMENTS

Background
This study undertakes a systematic appraisal of the validity of the hypothesis of

xenobiotic-induced, micronutrient-deficient, oxidative-stress mediated cellular injury in chronic pancreatitis.

Research frontiers

This is the first modern systematic appraisal of the validity of this hypothesis. The study also sets the available evidence in the context of current knowledge of the pathobiology of chronic pancreatitis.

Innovations and breakthroughs

Insight gleaned from this review is not innovative but questions the validity of the concept of xenobiotic exposure and subsequent cytochrome P450 induction in the aetiology of chronic pancreatitis.

Applications

The weakness of this hypothesis explains the negative results of the better-designed and executed studies of antioxidant therapy in chronic pancreatitis.

Terminology

Current terminology on chronic pancreatitis is utilised throughout.

Peer review

The authors reviewed the related researches and discussed the rationality of it, especially the necessary of antioxidants medication. This is an interesting review on pathogenesis of chronic pancreatitis.

REFERENCES

1   **Braganza JM**, Dormandy TL. Micronutrient therapy for chronic pancreatitis: rationale and impact. *JOP* 2010; **11**: 99-112 [PMID: 20208316]

2   **Wallig MA**. Xenobiotic metabolism, oxidant stress and chronic pancreatitis. Focus on glutathione. *Digestion* 1998; **59** Suppl 4: 13-24 [PMID: 9832632 DOI: 10.1159/000051439]

3   **Lu SC**. Regulation of glutathione synthesis. *Mol Aspects Med* 2009; **30**: 42-59 [PMID: 18601945 DOI: 10.1016/j.mam.2008.05.005]

4   **Guyan PM**, Uden S, Braganza JM. Heightened free radical activity in pancreatitis. *Free Radic Biol Med* 1990; **8**: 347-354 [PMID: 2379863 DOI: 10.1016/0891-5849(90)90100-W]

5   **Uden S**, Bilton D, Nathan L, Hunt LP, Main C, Braganza JM. Antioxidant therapy for recurrent pancreatitis: placebo-controlled trial. *Aliment Pharmacol Ther* 1990; **4**: 357-371 [PMID: 2103755 DOI: 10.1111/j.1365-2036.1990.tb00482.x]

6   **Uden S**, Schofield D, Miller PF, Day JP, Bottiglier T, Braganza JM. Antioxidant therapy for recurrent pancreatitis: biochemical profiles in a placebo-controlled trial. *Aliment Pharmacol Ther* 1992; **6**: 229-240 [PMID: 1600043 DOI: 10.1111/j.1365-2036.1992.tb00266.x]

7   **Segal I**. Pancreatitis in Soweto, South Africa. Focus on alcohol-related disease. *Digestion* 1998; **59** Suppl 4: 25-35 [PMID: 9832633 DOI: 10.1159/000051440]

8   **McCloy R**. Chronic pancreatitis at Manchester, UK. Focus on antioxidant therapy. *Digestion* 1998; **59** Suppl 4: 36-48 [PMID: 9832634 DOI: 10.1159/000051441]

9   **De las Heras Castaño G**, García de la Paz A, Fernández MD, Fernández Forcelledo JL. Use of antioxidants to treat pain in chronic pancreatitis. *Rev Esp Enferm Dig* 2000; **92**: 375-385 [PMID: 10985097]

10 **Uomo G**, Talamini G, Rabitti PG. Antioxidant treatment in hereditary pancreatitis. A pilot study on three young patients. *Dig Liver Dis* 2001; **33**: 58-62 [PMID: 11303976 DOI: 10.1016/S1590-8658(01)80136-5]

11 **Kirk GR**, White JS, McKie L, Stevenson M, Young I, Clements WD, Rowlands BJ. Combined antioxidant therapy reduces pain and improves quality of life in chronic pancreatitis. *J Gastrointest Surg* 2006; **10**: 499-503 [PMID: 16627214 DOI: 10.1016/j.gassur.2005.08.035]

12 **Siriwardena AK**, Mason JM, Sheen AJ, Makin AJ, Shah NS. Antioxidant therapy does not reduce pain in patients with chronic pancreatitis: the ANTICIPATE study. *Gastroenterology* 2012; **143**: 655-663.e1 [PMID: 22683257 DOI: 10.1053/j.gastro.2012.05.046]

13 **Fitzsimmons D**, Kahl S, Butturini G, van Wyk M, Bornman P, Bassi C, Malfertheiner P, George SL, Johnson CD. Symptoms and quality of life in chronic pancreatitis assessed by structured interview and the EORTC QLQ-C30 and QLQ-PAN26. *Am J Gastroenterol* 2005; **100**: 918-926 [PMID: 15784041 DOI: 10.1111/j.1572-0241.2005.40859.x]

14 **Braganza JM**. Limitations of patient selection and other issues in chronic pancreatitis antioxidant trial. *Gastroenterology* 2013; **144**: e17-e18 [PMID: 23357060 DOI: 10.1053/j.gastro.2012.12.034]

15 **Bhardwaj P**, Garg PK, Maulik SK, Saraya A, Tandon RK, Acharya SK. A randomized controlled trial of antioxidant supplementation for pain relief in patients with chronic pancreatitis. *Gastroenterology* 2009; **136**: 149-159.e2 [PMID: 18952082 DOI: 10.1053/j.gastro.2008.09.028]

16 **EUROPAC**. The European Registry of hereditary pancreatitis and familial pancreatic cancer. Available from: URL: http://www.europac-org.eu

17 **McNamee R**, Braganza JM, Hogg J, Leck I, Rose P, Cherry NM. Occupational exposure to hydrocarbons and chronic pancreatitis: a case-referent study. *Occup Environ Med* 1994; **51**: 631-637 [PMID: 7951796 DOI: 10.1136/oem.51.9.631]

18 **Jeppe CY**, Smith MD. Transversal descriptive study of xenobiotic exposures in patients with chronic pancreatitis and pancreatic cancer. *JOP* 2008; **9**: 235-239 [PMID: 18326937]

19 **Segal I**, Ally R, Hunt LP, Sandle LN, Ubbink JB, Braganza JM. Insights into the development of alcoholic chronic pancreatitis at Soweto, South Africa: a controlled cross-sectional study. *Pancreas* 2011; **40**: 508-516 [PMID: 21499204 DOI: 10.1097/MPA.0b013e318214f94a]

20 **Foster JR**, Idle JR, Hardwick JP, Bars R, Scott P, Braganza JM. Induction of drug-metabolizing enzymes in human pancreatic cancer and chronic pancreatitis. *J Pathol* 1993; **169**: 457-463 [PMID: 8501544 DOI: 10.1002/path.1711690412]

21 **Wacke R**, Kirchner A, Prall F, Nizze H, Schmidt W, Fischer U, Nitschke FP, Adam U, Fritz P, Belloc C, Drewelow B. Up-regulation of cytochrome P450 1A2, 2C9, and 2E1 in chronic pancreatitis. *Pancreas* 1998; **16**: 521-528 [PMID: 9598815 DOI: 10.1097/00006676-199805000-00011]

22 **Standop J**, Schneider M, Ulrich A, Büchler MW, Pour PM. Differences in immunohistochemical expression of xenobiotic-metabolizing enzymes between normal pancreas, chronic pancreatitis and pancreatic cancer. *Toxicol Pathol* 2003; **31**: 506-513 [PMID: 14692619]

23 **Pavek P**, Dvorak Z. Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochrome P450 superfamily in human extrahepatic tissues. *Curr Drug Metab* 2008; **9**: 129-143 [PMID: 18288955 DOI: 10.2174/138920008783571774]

24 **Apte MV**, Pirola RC, Wilson JS. Mechanisms of alcoholic pancreatitis. *J Gastroenterol Hepatol* 2010; **25**: 1816-1826 [PMID: 21091991 DOI: 10.1159/000090170]

25 **Nelson DR**. The cytochrome p450 homepage. *Hum Genomics* 2009; **4**: 59-65 [PMID: 19951895]

26 **Zordoky BN**, El-Kadi AO. Role of NF-kappaB in the regulation of cytochrome P450 enzymes. *Curr Drug Metab* 2009; **10**: 164-178 [PMID: 19275551 DOI: 10.2174/138920009787522151]

27 **Burim RV**, Canalle R, Martinelli Ade L, Takahashi CS. Polymorphisms in glutathione S-transferases GSTM1, GSTT1 and GSTP1 and cytochromes P450 CYP2E1 and CYP1A1 and susceptibility to cirrhosis or pancreatitis in alcoholics. *Mutagenesis* 2004; **19**: 291-298 [PMID: 15215328 DOI: 10.1093/mutage/geh034]

28 **Wallace K**, Marek CJ, Currie RA, Wright MC. Exocrine pancreas trans-differentiation to hepatocytes--a physiological response to elevated glucocorticoid in vivo. *J Steroid Biochem Mol Biol* 2009; **116**: 76-85 [PMID: 19446026 DOI: 10.1016/j.jsbmb.2009.05.002]

29 **Ulrich AB**, Standop J, Schmied BM, Schneider MB, Lawson TA, Pour PM. Species differences in the distribution of drug-metabolizing enzymes in the pancreas. *Toxicol Pathol* 2002; **30**: 247-253 [PMID: 11950168 DOI: 10.1080/019262302753559

588]

30 **Bruce JI**, Elliott AC. Pharmacological evaluation of the role of cytochrome P450 in intracellular calcium signalling in rat pancreatic acinar cells. *Br J Pharmacol* 2000; **131**: 761-771 [PMID: 11030726 DOI: 10.1038/sj.bjp.0703631]

31 **Mankad P**, James A, Siriwardena AK, Elliott AC, Bruce JI. Insulin protects pancreatic acinar cells from cytosolic calcium overload and inhibition of plasma membrane calcium pump. *J Biol Chem* 2012; **287**: 1823-1836 [PMID: 22128146 DOI: 10.1074/jbc.M111.326272]

32 **Hayes JD**, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol* 1995; **30**: 445-600 [PMID: 8770536 DOI: 10.3109/10409239509083491]

33 **Ulrich AB**, Schmied BM, Matsuzaki H, Lawson TA, Friess H, Andrén-Sandberg A, Büchler MW, Pour PM. Increased expression of glutathione S-transferase-pi in the islets of patients with primary chronic pancreatitis but not secondary chronic pancreatitis. *Pancreas* 2001; **22**: 388-394 [PMID: 11345140 DOI: 10.1097/00006676-200105000-00009]

34 **Milnerowicz H**, Jabłonowska M, Bizoń A. Change of zinc, copper, and metallothionein concentrations and the copper-zinc superoxide dismutase activity in patients with pancreatitis. *Pancreas* 2009; **38**: 681-688 [PMID: 19629005 DOI: 10.1097/MPA.0b013e3181a53d1]

35 **Hausmann DH**, Porstmann T, Weber I, Hausmann S, Dummler W, Liebe S, Emmrich J. Cu/Zn-SOD in human pancreatic tissue and pancreatic juice. *Int J Pancreatol* 1997; **22**: 207-213 [PMID: 9444552]

36 **Rahman SH**, Nanny C, Ibrahim K, O'Reilly D, Larvin M, Kingsnorth AJ, McMahon MJ. Genetic polymorphisms of GSTT1, GSTM1, GSTP1, MnSOD, and catalase in nonhereditary chronic pancreatitis: evidence of xenobiotic stress and impaired antioxidant capacity. *Dig Dis Sci* 2005; **50**: 1376-1383 [PMID: 16047490 DOI: 10.1007/s10620-005-2790-7]

37 **Abu-Hilal M**, McPhail MJ, Marchand L, Johnson CD. Malondialdehyde and superoxide dismutase as potential markers of severity in acute pancreatitis. *JOP* 2006; **7**: 185-192 [PMID: 16525202]

38 **Wang X**, Vatamaniuk MZ, Roneker CA, Pepper MP, Hu LG, Simmons RA, Lei XG. Knockouts of SOD1 and GPX1 exert different impacts on murine islet function and pancreatic integrity. *Antioxid Redox Signal* 2011; **14**: 391-401 [PMID: 20586612 DOI: 10.1089/ars.2010.3302]

39 **Cuzzocrea S**, Genovese T, Mazzon E, Di Paola R, Muià C, Britti D, Salvemini D. Reduction in the development of cerulein-induced acute pancreatitis by treatment with M40401, a new selective superoxide dismutase mimetic. *Shock* 2004; **22**: 254-261 [PMID: 15316396 DOI: 10.1097/01.shk.0000132490.79498.11]

40 **Sandilands D**, Jeffrey IJ, Haboubi NY, MacLennan IA, Braganza JM. Abnormal drug metabolism in chronic pancreatitis. Treatment with antioxidants. *Gastroenterology* 1990; **98**: 766-772 [PMID: 2298375]

41 **Aghdassi AA**, Mayerle J, Christochowitz S, Weiss FU, Sendler M, Lerch MM. Animal models for investigating chronic pancreatitis. *Fibrogenesis Tissue Repair* 2011; **4**: 26 [PMID: 22133269]

42 **Westlund KN**, Vera-Portocarrero L. Rat models of pancreatitis pain. *Methods Mol Biol* 2012; **851**: 223-238 [PMID: 22351095 DOI: 10.1007/978-1-61779-561-9_17]

43 **Gómez JA**, Molero X, Vaquero E, Alonso A, Salas A, Malagelada JR. Vitamin E attenuates biochemical and morphological features associated with development of chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G162-G169 [PMID: 15001429 DOI: 10.1152/ajpgi.00333.2003]

44 **Yoo BM**, Oh TY, Kim YB, Yeo M, Lee JS, Surh YJ, Ahn BO, Kim WH, Sohn S, Kim JH, Hahm KB. Novel antioxidant ameliorates the fibrosis and inflammation of cerulein-induced chronic pancreatitis in a mouse model. *Pancreatology* 2005; **5**:

165-176 [PMID: 15849487 DOI: 10.1159/000085268]

45 **de Las Heras-Castaño G**, García-Unzueta MT, Domínguez-Diez A, Fernández-González MD, García-de la Paz AM, Mayorga-Fernández M, Fernández-Fernández F. Pancreatic fibrosis in rats and its response to antioxidant treatment. *JOP* 2005; **6**: 316-324 [PMID: 16006681]

46 **Mas MR**, Isik AT, Yamanel L, Inal V, Tasci I, Deveci S, Mas N, Comert B, Akay C. Antioxidant treatment with taurine ameliorates chronic pancreatitis in an experimental rat model. *Pancreas* 2006; **33**: 77-81 [PMID: 16804416 DOI: 10.1097/01.mpa.0000222316.74607.07]

47 **Lu XL**, Song YH, Fu YB, Si JM, Qian KD. Ascorbic acid alleviates pancreatic damage induced by dibutyltin dichloride (DBTC) in rats. *Yonsei Med J* 2007; **48**: 1028-1034 [PMID: 18159597 DOI: 10.3349/ymj.2007.48.6.1028]

48 **Wei L**, Yamamoto M, Harada M, Otsuki M. Treatment with pravastatin attenuates progression of chronic pancreatitis in rat. *Lab Invest* 2011; **91**: 872-884 [PMID: 21383674 DOI: 10.1038/labinvest.2011.41]

49 **Li XC**, Lu XL, Chen HH. α-Tocopherol treatment ameliorates chronic pancreatitis in an experimental rat model induced by trinitrobenzene sulfonic acid. *Pancreatology* 2011; **11**: 5-11 [PMID: 21311207 DOI: 10.1159/000309252]

50 **Jiang F**, Liao Z, Hu LH, Du YQ, Man XH, Gu JJ, Gao J, Gong YF, Li ZS. Comparison of antioxidative and antifibrotic effects of α-tocopherol with those of tocotrienol-rich fraction in a rat model of chronic pancreatitis. *Pancreas* 2011; **40**: 1091-1096 [PMID: 21926544 DOI: 10.1097/MPA.0b013e31821b59c6]

51 **Matsushita K**, Mizushima T, Shirahige A, Tanioka H, Sawa K, Ochi K, Tanimoto M, Koide N. Effect of taurine on acinar cell apoptosis and pancreatic fibrosis in dibutyltin dichloride-induced chronic pancreatitis. *Acta Med Okayama* 2012; **66**: 329-334 [PMID: 22918205]

52 **Forsmark CE**, Liddle RA. The challenging task of treating painful chronic pancreatitis. *Gastroenterology* 2012; **143**: 533-535 [PMID: 22841737 DOI: 10.1053/j.gastro.2012.07.029]

53 **Mason J**, Siriwardena AK. Designing future clinical trials in acute pancreatitis. *Pancreatology* 2005; **5**: 113-115 [PMID: 15849481 DOI: 10.1159/000085261]

54 National Information Center on Health Services Research and Health Care Technology (NICHSR). HTA 101: V. APPRAISING THE EVIDENCE. Accessed 7th May 2013. Available from: URL: http://www.nlm.nih.gov/nichsr/hta101/ta10107.html

55 **Braganza JM**, Jolley JE, Lee WR. Occupational chemicals and pancreatitis: a link? *Int J Pancreatol* 1986; **1**: 9-19 [PMID: 3320224]

56 **González AM**, Garcia T, Samper E, Rickmann M, Vaquero EC, Molero X. Assessment of the protective effects of oral tocotrienols in arginine chronic-like pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2011; **301**: G846-G855 [PMID: 21852363 DOI: 10.1152/ajpgi.00485.2010]

57 **Hackert T**, Werner J. Antioxidant therapy in acute pancreatitis: experimental and clinical evidence. *Antioxid Redox Signal* 2011; **15**: 2767-2777 [PMID: 21834688 DOI: 10.1089/ars.2011.4076]

58 **Yadav D**, Lowenfels AB. The epidemiology of pancreatitis and pancreatic cancer. *Gastroenterology* 2013; **144**: 1252-1261 [PMID: 23622135 DOI: 10.1053/j.gastro.2013.01.068]

59 **Hirota M**, Shimosegawa T, Masamune A, Kikuta K, Kume K, Hamada S, Kihara Y, Satoh A, Kimura K, Tsuji I, Kuriyama S. The sixth nationwide epidemiological survey of chronic pancreatitis in Japan. *Pancreatology* 2012; **12**: 79-84 [PMID: 22487515 DOI: 10.1016/j.pan.2012.02.005]

60 **Lankisch PG**, Assmus C, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic diseases in Lüneburg County. A study in a defined german population. *Pancreatology* 2002; **2**: 469-477 [PMID: 12378115 DOI: 10.1159/000064713]

61 **Johnson CD**, Hosking S. National statistics for diet, alcohol consumption, and chronic pancreatitis in England and Wales, 1960-88. *Gut* 1991; **32**: 1401-1405 [PMID: 1752477 DOI:

10.1136/gut.32.11.1401]

62   **Whitcomb DC**, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD. Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 1996; **14**: 141-145 [PMID: 8841182 DOI: 10.1038/ng1096-141]

63   **LaRusch J**, Whitcomb DC. Genetics of pancreatitis. *Curr Opin Gastroenterol* 2011; **27**: 467-474 [PMID: 21844754 DOI: 10.1097/MOG.0b013e328349e2f8]

64   **Whitcomb DC**. Genetic aspects of pancreatitis. *Annu Rev Med* 2010; **61**: 413-424 [PMID: 20059346 DOI: 10.1146/annurev.med.041608.121416]

65   **Whitcomb DC**, LaRusch J, Krasinskas AM, Klei L, Smith JP, Brand RE, Neoptolemos JP, Lerch MM, Tector M, Sandhu BS, Guda NM, Orlichenko L, Alkaade S, Amann ST, Anderson MA, Baillie J, Banks PA, Conwell D, Coté GA, Cotton PB, DiSario J, Farrer LA, Forsmark CE, Johnstone M, Gardner TB, Gelrud A, Greenhalf W, Haines JL, Hartman DJ, Hawes RA, Lawrence C, Lewis M, Mayerle J, Mayeux R, Melhem NM, Money ME, Muniraj T, Papachristou GI, Pericak-Vance MA, Romagnuolo J, Schellenberg GD, Sherman S, Simon P, Singh VP, Slivka A, Stolz D, Sutton R, Weiss FU, Wilcox CM, Zarnescu NO, Wisniewski SR, O'Connell MR, Kienholz ML, Roeder K, Barmada MM, Yadav D, Devlin B. Common genetic variants in the CLDN2 and PRSS1-PRSS2 loci alter risk for alcohol-related and sporadic pancreatitis. *Nat Genet* 2012;

44: 1349-1354 [PMID: 23143602 DOI: 10.1038/ng.2466]

66   **Erkan M**, Adler G, Apte MV, Bachem MG, Buchholz M, Detlefsen S, Esposito I, Friess H, Gress TM, Habisch HJ, Hwang RF, Jaster R, Kleeff J, Klöppel G, Kordes C, Logsdon CD, Masamune A, Michalski CW, Oh J, Phillips PA, Pinzani M, Reiser-Erkan C, Tsukamoto H, Wilson J. StellaTUM: current consensus and discussion on pancreatic stellate cell research. *Gut* 2012; **61**: 172-178 [PMID: 22115911]

67   **Ceyhan GO**, Demir IE, Rauch U, Bergmann F, Müller MW, Büchler MW, Friess H, Schäfer KH. Pancreatic neuropathy results in "neural remodeling" and altered pancreatic innervation in chronic pancreatitis and pancreatic cancer. *Am J Gastroenterol* 2009; **104**: 2555-2565 [PMID: 19568227 DOI: 10.1038/ajg.2009.380]

68   **Bouwense SA**, Olesen SS, Drewes AM, Frøkjær JB, van Goor H, Wilder-Smith OH. Is altered central pain processing related to disease stage in chronic pancreatitis patients with pain? An exploratory study. *PLoS One* 2013; **8**: e55460 [PMID: 23405154 DOI: 10.1371/journal.pone.0055460]

69   **Burton F**, Alkaade S, Collins D, Muddana V, Slivka A, Brand RE, Gelrud A, Banks PA, Sherman S, Anderson MA, Romagnuolo J, Lawrence C, Baillie J, Gardner TB, Lewis MD, Amann ST, Lieb JG, O'Connell M, Kennard ED, Yadav D, Whitcomb DC, Forsmark CE. Use and perceived effectiveness of non-analgesic medical therapies for chronic pancreatitis in the United States. *Aliment Pharmacol Ther* 2011; **33**: 149-159 [PMID: 21083584 DOI: 10.1111/j.1365-2036.2010.04491.x]

9 771007 932045