Revised version of GASTRO-D-12-0002R5 (MAY 2012).

Antioxidant Therapy does not reduce pain in patients with Chronic Pancreatitis: The ANTICIPATE study.

Short title: Antioxidant therapy for chronic pancreatitis.

Trial Registration: ISRCTN-21047731

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Disclosures: None of the authors have any known conflicts of interest (financial, professional or personal) in relation to this work.

Financial support: This study was supported by an unrestricted academic grant from Pharma Nord, Morpeth, UK.

The corresponding author had full access to all of the data and takes full responsibility for the veracity of the data and statistical analysis.

Author contributions

Author	Concept/ design	Funding	Data acquisition	Analysis	Drafting	Review of final manuscript
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Abstract

Background and aims: We investigated whether antioxidant therapy reduces pain and improves quality of life in patients with chronic pancreatitis**.**

Methods: We performed a double-blind, randomized controlled trial that compared the effects of antioxidant therapy with placebo in 70 patients with chronic pancreatitis. Patients provided 1 month of baseline data and were followed for 6 months while receiving either Antox version1.2 or matched placebo (2 tablets, 3 times daily). The primary analysis was baseline-adjusted change in pain score at 6 months, assessed by an 11-point numerical rating scale. Secondary analyses included alternative analyses of clinic and diary pain scores, scores on quality of life tests (the EORTC-QLQ-C30, QLQ-PAN28, EuroQOL EQ-5D, and EQ-VAS), levels of antioxidants, use of opiates, and adverse events. Analyses, reported by intention to treat, were prospectively protocol-defined.

Results: After 6 months, pain scores reported to the clinic were reduced by 1.97 from baseline in the placebo group and by 2.33 in the antioxidant group but were similar between groups (-0.36, 95%CI: -1.44 to 0.72, p=0.509). Average daily pain scores from diaries were also similar (3.05 for the placebo group, 2.93 for the antioxidant group, a difference of 0.11; 95% CI, 1.05–0.82; *P*=0.808). Measures of quality of life were similar between groups, as was opiate use and numbers of hospital admissions and outpatient visits. Blood levels of vitamin C and E, -carotene, and selenium were significantly increased in the antioxidant group**.**

Conclusions: In patients with painful chronic pancreatitis of predominantly alcoholic origin, antioxidant therapy did not reduce pain or improve quality of life, despite causing a sustained increase in blood levels of antioxidants.

Key words: randomized clinical trial; pancreatitis therapy; treatment response; efficacy

Introduction

Chronic pancreatitis (CP) is an inflammatory condition of the pancreas characterized histologically by loss of normal pancreatic parenchymal architecture with varying degrees of fibrosis and inflammatory infiltrate[1]. Clinically, chronic pancreatitis presents as a spectrum of disease characterized typically by chronic, unremitting and incapacitating abdominal pain together with varying features of pancreatic exocrine deficiency, which may lead to steatorrhea and manifestations of endocrine deficiency - diabetes mellitus[2].

To date there is no specific therapy for chronic pancreatitis. Surgical interventions can be grouped as either resectional (removing the diseased head or entire gland) or drainage procedures[3] (aimed at internal drainage of the dilated main pancreatic duct). An alternative strategy involves thoracoscopic division of splanchnic nerves[4]. Duct drainage can also be achieved endoscopically[5]. None of these procedures are universally applicable to all patients with chronic pancreatitis and they carry varying degrees of risk of failure to achieve sustained pain relief.

Seeking an alternative paradigm for chronic pancreatitis, Braganza and colleagues proposed that the disease arose as a result of pathological exposure of the acinar cells to short-lived oxygen free radicals – a process termed oxidative stress[6]. A deficient free radical quenching system combined with excess free radical production led to cellular injury[6]. Support for this hypothesis comes from several different sources: oxidative stress-response genes are up-regulated during experimental pancreatitis[7]; intra-vital microscopy using intra-acinar labeling has demonstrated short-lived oxidative bursts[8]; polymorphisms of the glutathione transferase gene are more prevalent in patients with chronic pancreatitis[9]; and, analysis of peripheral blood samples taken in clinical chronic pancreatitis have shown that anti-oxidants (inhibitors of the oxidative stress response), their precursors and co-factors in physiologic anti-oxidant pathways are depleted[10]. In addition, there is elevation of peripheral blood markers of oxidative injury[10]. These clinical findings have been reproduced in Sowetan Africans (a group with a high incidence of chronic pancreatitis) suggesting that the results are independent of race and geography[11].

Braganza and colleagues reasoned that exogenous supplementation with antioxidants or precursors for antioxidant pathways might help to reduce on-going acinar injury[12]. From a series of exploratory studies they concluded that co-factors of the endogenous glutathione peroxidase pathway were key components for supplementation. Selenium, vitamin C (ascorbic acid) and methionine were proposed as key antioxidants[13].

After two small randomized trials[12,14] of selenium, β-carotene, vitamins C & E and methionine-based antioxidant therapy reported a reduction in severity and frequency of episodes of pain in patients with recurrent and chronic pancreatitis, a commercially available formulation, Antox (Pharma Nord, Morpeth, UK) was developed comprising vitamin C, vitamin E, β-carotene, selenium and methionine. Despite the obvious attraction of a pharmacologic intervention, antioxidant therapy for chronic pancreatitis has not become accepted as standard therapy. The small, heterogeneous clinical trial base was thought to be a main reason for the lack of acceptance. The recent publication of a report from Delhi in which 147 patients were randomized to antioxidant therapy or placebo and which reported a main outcome measure of reduction in "painful days" might alter the position of equipoise[15]. However, the Delhi study population comprised mainly young patients (age 29.6 ± 9.3 sd [standard deviation] years in the placebo group and 31.3 ± 11.4 sd years in the antioxidant group) in whom only 40 had alcohol related disease compared to 87 with idiopathic chronic pancreatitis. Thus their recruited patients were very different to the older, alcohol-etiology dominant disease phenotype typically seen in Europe and the United States of America. Furthermore, the study undertook no formal quality of life analysis.

More than thirty years after the proposal of micronutrient antioxidant therapy for painful chronic pancreatitis the treatment remains only sporadically used and the optimal formulation of antioxidant regimen poorly understood. Given the dearth of alternative therapies for patients with chronic pancreatitis there was a pressing case for a well-designed study to evaluate the effect of antioxidant therapy in a clearly defined population of patients. Given the fluctuating course of this disease, an assessment of the effect of intervention on both pain and quality of life was needed, providing the rationale for the ANTICIPATE trial.

Methods

Study design

A double-blind, placebo-controlled, single-centre randomised trial of Antox version 1.2 (Pharma Nord, Morpeth, UK) in patients with painful chronic pancreatitis.

Setting

Tertiary care academic medical centre*.*

Hypotheses

This trial tested the primary hypothesis that antioxidant therapy with antox version 1.2 would reduce pain in patients with painful chronic pancreatitis. A secondary and supportive hypothesis was that treatment of these patients with Antox version 1.2 would improve quality of life as measured by validated questionnaires.

Definitions of chronic pancreatitis and patient assessment protocols

The terminology advocated by the Zurich international workshop was used to define chronic pancreatitis[16]. The etiology of CP was categorized as alcoholic, hyperlipidemic, familial or idiopathic. Patients underwent a detailed clinical, radiological and biochemical baseline assessment prior to enrolment. In addition to demographic data, specific information was collected on cigarette smoking, alcohol consumption, opiate intake and history of prior surgery or endoscopic intervention. After clinical assessment, full blood count, serum urea, electrolytes, biochemical liver function tests and lipid profiles were assessed together with fasting glucose, glycosylated hemoglobin and plasma CA 19-9. Baseline fasting anti-oxidant levels measured were: selenium, glutathione, vitamin C, vitamin E and β-carotene along with 9/11:9/12 linoleic acid ratio as a marker of free radical damage. Baseline assessments further included: body mass index (BMI), diabetic status (according to WHO 2006 criteria)[17] and fecal elastase (laboratory threshold of <200 μg/g stool diagnostic of exocrine insufficiency). A qualified dietitian assessed nutritional status. Imaging studies included: intravenous contrast-enhanced spiral contrast tomography (CT), endoscopic retrograde cholangiopancreatography (ERCP) in selected patients and magnetic resonance pancreatography (MRCP). ERCP/MRCP findings were graded from

1 to 4 (equivocal to marked) using the Cambridge criteria[18]. Cross-sectional pancreatic imaging was reviewed at an appropriate hepato-pancreato-biliary (HPB) multi-disciplinary meeting.

All patients received standard treatment for chronic pancreatitis (including analgesia as required) at the discretion of the clinical team providing care. All concurrent medication was recorded and non-protocol use of antioxidant therapy was specifically excluded.

Inclusion criteria:

These were: ability to give informed consent; age over 18 years; recent CT (ideally within 3 months of trial enrolment); either CT and/or ERCP or MRCP evidence of chronic pancreatitis; and a baseline daily pain score of 5 or greater on a numerical rating scale (NRS, scoring 0-10)[19] for at least 7 days in a pre-randomization run-in period of one month.

Exclusion criteria:

These were: inability to give informed consent, inability to comprehend or comply with the trial protocol, patients with chronic renal failure (with a creatinine clearance of less than 50 ml/minute), patients who were pregnant or lactating or who planned to become pregnant during the study period, those who were participating in another trial, patients who were already taking antioxidants and patients with a psychiatrist's diagnosis of schizophrenia. Patients with pancreatic cancer were also excluded.

Randomization, stratification and blinding

Computer generated randomization charts were produced by the statistician (JMM) who was not associated with the conduct of the study. Randomization was stratified by whether or not patients had undergone prior therapeutic pancreatic intervention (endoscopic, radiologic or surgical) and used a block size of four. Randomization charts were administered by the hospital trial pharmacy in a process ensuring concealed allocation. To provide double-blinding, drug and placebo were supplied by pharmacy as yellow, ovoid, compressed, film-coated tablets of similar appearance in sealed packages. Thus clinicians were separated from the randomization process and remained blind to the treatment allocated to patients. Similarly, patients remained blind to the treatment allocated.

Unblinding and withdrawal

In an emergency, treatment allocation could be unblinded at the request of the clinician providing care. Patients could withdraw from the trial at any point without alteration in standard care. The patient could be withdrawn if their attending clinician judged that circumstances arose that were detrimental to the individual.

Study medication

All patients recruited to the study received antioxidant therapy or matched placebo for the 6 month period of the trial. Trial packs contained 600 tablets as 20 blister strips of 30 Antox version 1.2 tablets or matched placebo, two tablets taken three times daily. Antioxidant supplementation contained active ingredients: 38.5 mg selenium yeast of which 50μg was l-selenomethionine, 113.4 mg d-α-tocopherol acetate, 126.3 mg ascorbic acid and 480 mg l-methionine together with secondary ingredients: 285.6 mg microcrystalline cellulose, 14.0 mg croscarmellose sodium, 7.0 mg colloidal anhydrous silica and 3.0 mg magnesium stearate. The coating included 4.2 mg β carotene. Placebo supplementation contained: 657.9 mg microcrystalline cellulose, 73.3 mg croscarmellose sodium, 15.0 mg colloidal anhydrous silica and 3.7 mg magnesium stearate per tablet.

Primary Outcome Measure

Change in clinic pain score from baseline to 6 months. The primary analysis compared the change in pain between groups, analysed by Student's t-test.

Secondary Outcome Measures

These included: clinic and diary pain scores as repeated measures; diary recorded pain analysed as the average of daily scores over 6 months; Brief Pain Inventory (BPI) scores analysed as repeated measures; quality of life scores analysed as repeated measures (EORTC-QLQC[20], QLQ-PAN26, EuroQOL[21] EQ-5D and EQ-VAS); change in antioxidant levels from baseline to 6 months; opiate usage (defined as morphine equivalents) analysed as repeated measures; rates of hospital admission for pancreatitis-related exacerbations or complications; and, rates of treatment-related side effects and complications.

Assessment of outcome measures and monitoring during study

The primary outcome measure was clinic pain score recorded using an 11-point numerical pain rating scale (NRS) previously validated for the assessment of chronic pain[19]. Patients were shown the scale by the research fellow during the consultation and asked to indicate where they felt their pain score should be marked. The same 11-point numerical pain rating scale was used by patients to record pain in a daily pain diary: this diary was maintained by the patients. Diary records were aggregated to average monthly scores from baseline (the month before randomization) to month 6. Clinic assessments (at 0, 2, 4 and 6 months) included: pain NRS scores, record of adverse events, the Brief Pain Inventory, disease specific quality of life measures: EORTC QLQ-C30 (score 30 to 120) and QLQ-PAN28 (score 26 to 112)[20] and generic measures: EuroQOL EQ-5D (score 0 to 1, negative scores possible) and EQ-VAS (Visual Analog Score, score 0 to 100)[21]; record of opiate use and hospitalization. Blood antioxidant levels were measured at baseline, study mid-point and at 6 months. Questionnaires were administered by the trial clinical research fellow (NS) in an outpatient clinic setting. Assessment of compliance was not formally tested in this pragmatic, outpatient based study design. However, completion of pain diaries and measurement of antioxidant levels were accepted as surrogate markers of compliance

Safety evaluation and reporting of adverse events

Patients were monitored for treatment-related side effects and complications. Adverse events were recorded, assessed for severity and attribution, and reported in line with European Directive 2001/20/EC. Specific pancreatitis-related complications included hospital admission with acute exacerbation of chronic pancreatitis or for pain control (defined from hospital discharge notes). Complications such as pancreatic pseudocyst or pancreatic abscess were defined according to the 1992 Atlanta consensus conference criteria[22].

Statistical Analysis

Sample size calculation. In a previously published study, anti-oxidant therapy was associated with a 12 point reduction in pain on a 100 point VAS (visual analog scale)[23]. The variance structure for a change in pain score in a cohort of patients with chronic pancreatitis was unknown, typical published values for VAS change scores in other populations suggested a standard deviation of 15 points. A sample size calculation based on a 1.2 point change in pain on a numerical rating scale (0-10, 11 points) with a standard deviation of 1.5 points, with 80% power and alpha at 0.05 required 26 patients in each arm of the trial. Allowing for 10% loss to follow-up, the trial aimed to recruit 57 patients. At an interim inspection, the independent trial steering committee advised that enrolment be increased to approximately 90 patients to accommodate effects of withdrawal or loss to follow-up.

Descriptive and analytic statistics. Descriptive statistics (continuous: mean and standard deviation; binary: proportion; categorical: median and range) were calculated for study variables. Differences between treatment groups for endpoint and change scores were analyzed using the unpaired Student t test; repeated measures analyses of variance were covariate-adjusted for the baseline measure and trial strata, matching the trial design; proportions were analyzed using exact tests; individual items of the EORTC questionnaires were analyzed using the Mann Whitney U test. EORTC total and sub-domain scores were analyzed as continuous measures consistent with the assumptions underpinning aggregation of responses to individual questions. Diary pain scores were averaged monthly and over the entire 6 month follow-up period. Since hospital admission data are typically highly skewed, estimates of differences used bootstrapping with 10,000 samples. All analyses were conducted by

intention to treat and to protocol. SPSS 19 (IBM, New York, USA) was used for statistical analysis.

Ethical Approvals

The study protocol was approved by the North-West Regional Ethical committee (MREC, 07/MRE08/13) and the United Kingdom Medicines & Health products Regulatory Agency (MHRA, 2006-006958-10). Study oversight was provided by an independent trial steering committee.

The study was registered with the International Standard Randomized Controlled Trial database (ISRCTN-21047731). Trial reporting follows CONSORT guidelines (Figure 1).

RESULTS

In total, 356 patients with CP were assessed during the enrolment period from February 2008 to August 2009, of whom 92 fulfilled the inclusion criteria and agreed to participate in the trial.

Completeness of follow-up

One patient randomized to placebo withdrew and did not receive intervention. Fifteen patients withdrew within the first two months of enrolment. Six further patients were lost to follow-up after completion of the first 2 months but before the 6 month appointment. No patients were withdrawn by the investigators. Seventy patients completed follow-up to six months and are reported in the analysis (see Figure 1). The study was closed to new recruitment at the end of August 2009 with a subsequent 6 month data maturation phase to allow for completion of data collection.

Baseline comparability

Treatment and placebo groups were similar at baseline with respect to age, gender, duration of disease, previous intervention, etiology of chronic pancreatitis, pancreatic exocrine function, alcohol and cigarette consumption, BMI, diabetes and opiate use (Table 1).

Response to treatment

Primary outcome measure.

NRS pain scores reported on the day of clinic visits are shown in Figure 2. Analyses of change scores show a reduction in pain in both groups from baseline to 6 months, but no statistically significant difference in reduction (Placebo: -1.97, Antioxidant: -2.33, Difference: -0.36, , 95%CI: -1.44 to 0.72, p=0.509, see Table 2).

Re-analysis of clinic pain scores using repeated measures ANOVA supported the primary analysis of no difference in pain between antioxidant and placebo groups (-0.07, 95%CI: -0.97 to 0.83, p=0.875;Table 2 and Figure 2).

Secondary outcome measures.

Analysis of diary data found no difference in pain between antioxidant and placebo groups as a change score (-0.34, 95%CI: -0.98 to 0.31, p=0.302), using repeated measures ANOVA (0.04, 95%CI: -0.61 to 0.52, p=0.878) or daily average score (-0.11, 95%CI: -1.05 to 0.82, p=0.808), supporting the primary analysis (Table 2 and Figure 3).

The Brief Pain Inventory addresses the location of disease specific pain, assessing worst, least and average levels of pain in the preceding week: analyses of these three measures similarly found no difference in pain between groups (Table 2).

Quality of life scores were analysed as repeated measures. There was no evidence of differences in EORTC QLQ-C30, PAN28, EuroQOL EQ-5D or EQ-VAS as summary scores (Table 2). Analysis of individual questions and sub-domains of both QLQ-PAN28 and QLQ-C30 at six months identified no statistically significant differences.

Need for supportive care.

Average opiate usage was similar between groups and consistent over the 6 month period of follow-up. Similarly, pancreatitis-related hospital inpatient stays and outpatient clinic attendances were similar between groups (Table 2).

Antioxidant levels

Serum/plasma antioxidant levels (vitamin C, vitamin E, β-carotene, selenium) were increased significantly at 6 months in patients receiving active treatment, while placebo levels remained similar to baseline (Table 3). Measures of whole blood glutathione were not modified substantially within the trial.

Haematological and biochemical levels

Across a wide range of haematological and biochemical values, no significant differences emerged between groups during treatment (Table 3).

Adverse events

A total of 9 adverse events were recorded (1 placebo and 8 antioxidant). Of these, 7 events were mild and related to bad taste and heartburn with nausea. One moderately severe adverse event in the antioxidant group related to increased frequency of stool and occasional diarrhoea. The other moderately severe adverse event in the antioxidant group was a patient hospitalized with convulsions due to hepatic encephalopathy. Apart from this last individual the other 8 patients reporting adverse events withdrew within the first few months of follow-up.

Requirement for alternative interventions during the study

No patients underwent surgery for chronic pancreatitis during the course of the study. Two patients in the placebo group underwent endoscopic intervention: one underwent pancreatic duct stenting and the other underwent endoscopic drainage of a pancreatic pseudocyst. This latter individual was one of the patients who withdrew from the study. One further patient in the placebo arm underwent an urgent laparotomy for visceral perforation, temporarily withdrew from the study during the post-operative period, but then re-entered and completed his allocated intervention.

DISCUSSION

This trial addresses the question of the value of antioxidant therapy for patients with chronic pancreatitis. The answer is important since the health care burden of chronic pancreatitis is considerable and patients with this disease suffer from sustained distressing symptoms of which pain is the dominant feature. A pharmacologic intervention is attractive as it obviates the need for surgical or endoscopic interventions (neither of which is consistently beneficial in reducing pain).

Emphasis was placed within the trial design on the definition of the study population. The aim was to focus on those patients most typically seen in a European or North American Gastroenterology clinic in whom alcohol would be the predominant etiologic agent. Radiologic evidence of chronic pancreatitis was mandatory both to help diagnose and categorise chronic pancreatitis and to help exclude other conditions such as pancreatic cancer which can mimic chronic pancreatitis. In keeping with current specialist pancreatic practice, not all patients underwent ERCP and no patients underwent diagnostic ERCP. In United Kingdom practice, interventional tests of pancreatic endocrine function are not widespread and standard practice includes measurement of faecal elastase.

A second aspect of the study design is the selection of patients with stable but symptomatic disease. A daily NRS pain score of 5 or greater on at least 7 days during the one month pre-randomization run-in period was required for a patient to be enrolled. Conduct of the study at a single center might compromise the generalizability of the findings. However, the spectrum of disease was typical for a tertiary clinic and European or North American population, and use of a single researcher for clinic assessment facilitated consistent measurement of pain in patients. A further important issue is that of power. Post hoc re-examination of the variance in pain numerical rating scale scores from our own data suggests a more conservative standard deviation of 2 points rather than 1.5. A change of 1.2 points (or an effect size of 0.6) suggests a planned recruitment of 90 subjects and since we report only 70 patients there might be an argument that we cannot exclude type II error at conventional levels. Further, differences in assessment and reporting of pain in clinical studies of chronic pancreatitis ("painful days" used in the Bhardwaj study)[15] and also in other chronic disease states can make comparison between

studies difficult[19]. Thus it would be wise to accept that the possibility of type II error has not categorically been rejected.

The duration of intervention, use of a placebo arm and stratification are also features with potentially critical influence on trial outcome. A six-month follow-up period was thought to be of sufficient duration to be representative, allowing sufficient time for selenium levels to attain plateau but avoiding the potential ethical difficulties of giving patients placebo treatment for prolonged periods. A placebo arm was felt to be justifiable and necessary given the general lack of acceptance of antioxidant therapy in chronic pancreatitis. Patients were stratified by prior pancreatic intervention to design out an important potential confounding variable. It might be argued that end-stage pancreatic disease is another important confounder although difficult to stratify within the randomisation when occurring in small numbers. Proxied by faecal elastase measurement, randomisation allocated these patients similarly to treatment and placebo groups (see Table 1).

The primary endpoint was selected as the baseline-adjusted change in clinic pain score and supportive secondary endpoints included other analyses and assessments of pain, as well as quality of life using separate, validated assessment tools.

With these design features in mind, the present study represents a well-designed trial conducted in a relevant and well-characterised study population. The recruitment process delivered two groups similar in terms of age, gender, disease profile and duration, etiology of CP and opiate use (Table 1). Compliance with study drug use was not formally verified although plasma antioxidant levels in patients demonstrate consistent and considerable treatment effect consistent with compliance. Similarly, the 70 patients completing the study provided complete diary data suggesting considerable engagement with the study aims.

There was no significant difference between groups in any outcome measured other than change in antioxidant levels (Tables 2 and 3). Analyses of change of pain scores show a reduction in pain in both groups from baseline to 6 months but no significant difference between groups (Figure 2). These findings may help explain why individual patients and inadequately controlled studies might attribute benefit to antioxidant therapy, if receiving treatment in response to an episode of pain. Assessment of quality of life was necessary since pain has a diffuse impact on multiple aspects of individual

functioning. Comprehensive quality of life assessment showed that there were no reported benefits from antioxidant treatment in any of the domains assessed.

Of 356 patients assessed for eligibility, 92 patients entered the trial. The main reasons for exclusion were either that patients had already been initiated on, or were not eligible for, antioxidant therapy under normal clinical care. Thus there were no selection effects limiting the disease spectrum of patients participating in the trial. Patient decisions to withdraw or not to attend follow-up clinics led to 22 individuals being were lost to the analysis. Withdrawals were similar, by treatment allocation, in age, gender and baseline pain scores.

A previous trial of antioxidant treatment for chronic pancreatitis took as its primary definition: 'painful days per month' recorded within pain diaries[15]. This assessment was not performed directly in the present study but could be approximated with the data generated within this trial by setting a threshold on the diary VAS pain score qualifying as a "painful day". The threshold was varied from 1 to 10: at no threshold was there a significant difference in days free from pain comparing antioxidant and placebo (Figure 4).

Previous studies of antioxidant therapy in chronic pancreatitis can be dichotomized into several older, smaller trials[12, 14, 23,] and one more recent, well-designed larger trial[15]. Interpretation of the small trials is problematic due to their poorly characterized and heterogeneous patient populations (not all patients in the early Manchester studies had chronic pancreatitis) as well as non-standardised reporting of endpoints. In contrast, Bhardwaj and colleagues reported the largest randomized trial of antioxidant therapy in chronic pancreatitis with similar assessment and work-up protocols to the present study. The principle conclusion of Bhardwaj that "antioxidant supplementation was effective in relieving pain in patients with chronic pancreatitis" is in direct contrast to the findings of the present trial. A potential reason for this discrepancy could lie in differences in the patient cohorts recruited: the mean age was lower: 31 years compared to 50 years in the present study. Similarly, etiology differed: 31% vs. 72% of predominantly alcoholic origin; alcohol consumption was almost two times higher in the present study while Bhardwaj report a greater proportion of patients with malnutrition. Further, in the Bhardwaj study

there were 36 (28%) cigarette smokers compared to 56 (80%) in the present study. In addition to these clinical and demographic differences, there is a difference in the morphological disease profile: Bhardwaj reports that 79% of patients had evidence of a dilated main pancreatic duct compared to 57% in the present study. Finally the composition and dose of antioxidant constituents differs between the trials. While both study cohorts of patients might fulfil the broad criteria for definition of chronic pancreatitis, the present study provides a population of older, alcohol-etiology dominant disease that is typical of the clinical phenotype seen in Europe and North America. Interestingly, Bhardwaj reports no benefit (of reduced pain) in the sub-group of patients with alcoholic aetiology (antioxidant 3.7 painful days *vs* placebo 4.2 painful days; p=0.61), consistent with the general finding of the present study. Different methods of assessment might account for some discrepancy: the primary endpoint of "painful days" utilized by the Bhardwaj study was based solely on patient diary records. In the present study, a clinic-based estimate of pain using a validated numerical rating scale was the primary endpoint but was contextualised by diary-based pain scores and quality of life assessment.
Since there are some differences both in inclusion and assessment, replication of studies (with an extended range of outcomes drawn from both studies) may be required in order to provide definitive resolution of uncertainties.

How might the apparent lack of effect of antioxidant therapy be reconciled with the genetic evidence of up-regulation of oxidative stress response genes and the cell biological evidence of oxidative stress? A potentially important mechanistic insight from the present study is that exogenous dietary supplementation – in pharmacological concentrations – may cause a significant elevation in circulating antioxidant levels but not have an impact on symptoms. Thus, at the point of presentation with symptoms, with evidence of likely irreversible pancreatic parenchymal and functional alteration, the potential time-point for disease modification by exogenous antioxidant supplementation may be past.

In summary, this paper reports a randomized controlled trial of the compound antioxidant therapy Antox version 1.2 in a well-characterised population of patients with chronic pancreatitis with comparable clinical characteristics at baseline. The primary outcome measure of NRS pain scores reported on the day of clinic visits shows a reduction in pain in both groups from baseline to 6 months, but no

statistically significant difference in reduction (Placebo: -1.97, Antioxidant: -2.33, Difference: -0.36, p=0.509). Similarly there was no difference in diary-based visual analogue scores, Brief Pain Inventory or quality of life using validated questionnaires.

Despite the lack of a strong evidence base, micronutrient antioxidant therapy has for over 30 years been regarded as an alternative paradigm for chronic pancreatitis and continues to be strongly advocated by its supporters. In this regard the importance of the present study is that when patients present with abdominal pain, with clinical, radiological and physiological evidence of chronic pancreatitis, micronutrient antioxidant therapy with Antox is not likely to contribute to any reduction in pain or improvement in quality of life.

ACKNOWLEDGEMENTS

The authors acknowledge the contributions of the staff of the Pancreatic laboratory of the Manchester Royal Infirmary.

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Table 1. Baseline Demographic and Clinical Parameters: Antioxidant and Placebo Groups

	Placebo (37)	Antioxidant (33)	p
Age at enrolment (y)	50±9	49.8±12.7	0.96
Gender male:female	27:10	23:10	0.80
Disease duration (y)	4.9±4.3	4.2±2.4	0.36
Clinic NRS (SD)	5.0±1.6	5.2±1.6	0.36
Previous intervention yes:no	20:17	18:15	1.00
Etiology alcohol:idiopathic	27:10	24:9	1.00
ER (MR) CP*			0.19
Equivocal	0 (0%)	1 (6.3%)	
Mild	4 (16.7%)	4 (25.0%)	
Moderate	15 (62.5%)	9 (56.3%)	
Marked	5 (20.8%)	2 (12.5%)	
CT			0.13
Calcification	12 (32.4%)	18 (54.5%)	
Dilated pancreatic duct	2 (5.4%)	2 (6.1%)	
Calcification and dilated pancreatic duct	23 (62.2%)	13 (39.4%)	
Faecal elastase (μg/g)	192±198	221±198	0.56
Faecal elastase <15 (μg/g)	7	8	
Alcohol (g/d)	247±202	222±123	0.59
Cigarette smoker: yes:no	28:9	28:5	0.38
Cigarettes (/d)	22±8	21±11	0.83
BMI (kg/m)	22.7±4.5	23.2±3.8	0.62
Diabetes mellitus: yes:no	11:26	10:23	1.00
Morphine equivalent (mg/d)	91±105	85±114	0.84

* Data available for 24 placebo and 14 antioxidant receiving patients, **d**=day, **μg**=micro gram, **kg=** kilogram, g=gram, **y**=years.

Table 2. Analysis of response to treatment

	Placebo [P=37]	Antioxidant [A=33]	[A-P] (95%CI)	p
Pain scores				
Clinic NRS [change] (SD)[1]	-1.97 (2.46)	-2.33 (2.09)	-0.36 (-1.44 to 0.72)	0.509
Clinic NRS [R-M][2]	3.09	3.02	-0.07 (-0.97 to 0.83)	0.875
Diary NRS [change] (SD)[3]	-0.80 (1.35)	-1.14 (1.35)	-0.34 (-0.98 to 0.31)	0.302
Diary NRS [R-M][4]	2.97	3.02	0.04 (-0.61 to 0.52)	0.878
Diary NRS [average] (SD)[5]	3.05 (1.96)	2.93 (1.96)	-0.11 (-1.05 to 0.82)	0.808
BPI [worst, R-M][2,6]	4.16	4.47	0.31 (-0.99 to 01.62)	0.632
BPI [least, R-M][2,6]	1.83	1.79	-0.04 (-0.77 to 0.69)	0.913
BPI [average, R-M][2,6]	3.30	3.21	-0.09 (-1.00 to 0.84)	0.854
Quality of life measures				
EORTC QLQ-C30[2]	65.2	62.1	-3.3 (-9.4 to 2.8)	0.283
Physical functioning (Q. 1-5)	9.07	8.08	-0.99 (-2.20 to 0.22)	0.106
Role functioning (Q. 6,7)	4.27	4.19	-0.08 (-0.85 to 0.70)	0.844
Cognitive functioning (Q. 20,25)	3.88	3.53	-0.35 (-0.89 to 0.20)	0.208
Emotional functioning (Q. 21-24)	9.05	8.80	-0.25 (-1.46 to 0.97)	0.688
Social functioning (Q. 26,27)	4.18	4.02	-0.16 (-0.88 to 0.57)	0.667
Overall global quality of life (Q. 29,30)	8.15	8.53	-0.38 (-0.88 to 1.64)	0.548
EORTC QLQ-PAN28[2]	59.9	55.8	-4.1 (-8.5 to 0.2)	0.060
Pancreatic pain (Q. 31,33,35)	6.65	6.57	-0.08 (-1.05 to 0.90)	0.874
EQ-5D[2]	0.51	0.55	0.04 (-0.10 to 0.19)	0.559
EQ-VAS[2]	56.6	58.9	2.3 (-6.5 to 11.1)	0.601
Supportive care				
Morphine equivalent (mg/day)[2]	92.7	79.0	-13.7 (-38.0 to 10.6)	0.266
Hospital Inpatients (days)[7]	4.00 (8.11)	3.94 (7.75)	-0.06 (-3.80 to 3.53)	-
Hospital outpatient visits[7]	1.32 (1.25)	1.12 (1.27)	-0.20 (-0.78 to 0.38)	-

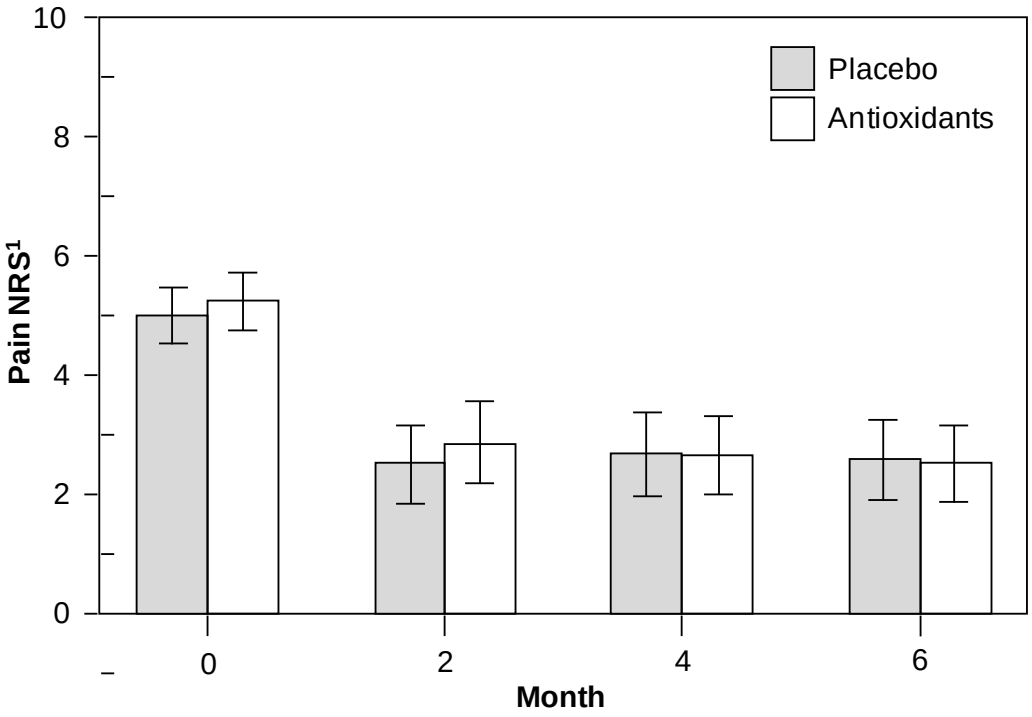
1 Change score (6 months – baseline)
2 Repeated measures model: estimated mean of observations at 2, 4 and 6 months
3 Change score (month 6 – month pre baseline)
4 Repeated measures model: estimated mean of observations for months 1 to 6
5 Average daily pain score during months 1 to 6.
6 Brief Pain Inventory: average level of pain in the previous week
7 Bootstrapped estimate of difference and confidence interval

Table 3. Changes in antioxidant, haematological and biochemical levels

	Placebo [P=37]	Antioxidant [A=33]	[A-P] (95%CI)	P
Antioxidant levels[1]				
Vitamin C (mg/mL)	-0.70 (5.15)	8.34 (8.76)	9.04 (5.54 to 12.54)	<0.001
Vitamin E[1] (mg/mL) [1]	-1.88 (10.02)	7.42 (17.95)	9.30 (2.20 to 16.40)	<0.011
β-carotene (mg/mL)[1]	7.85 (34.05)	62.56 (125.68)	54.72 (8.83 to 100.51)	0.021
Selenium (µmol/L)[1]	0.92 (12.39)	42.73 (32.27)	41.81 (29.73 to 53.88)	<0.001
WGSH[1]	-3.72 (176.91)	-32.38 (251.29)	-28.65 (-132.98 to 75.68)	0.593
WGSH-Hb[1]	-0.0028 (1.1783)	0.0212 (1.1671)	0.0240 (-0.5450 to 0.5929)	0.933
WGSH-RBC[1]	-86.94 (364.50)	-25.25 (377.97)	61.69 (-119.88 to 243.26)	0.500
Biochemistry values[11]				
Hemoglobin (gm/dL)	-0.211 (1.375)	-0.118 (1.305)	-0.093 (-0.732 to 0.547)	0.773
Glycosylated hemoglobin (%)	-0.084 (1.015)	0.030 (0.801)	-0.114 (-0.548 to 0.320)	0.602
White cell count (10^9/l)	-0.611 (2.331)	-0.570 (2.382)	-0.041 (-1.168 to 1.086)	0.942
Fasting blood glucose (mmol/L)	0.551 (2.671)	0.209 (2.988)	0.342 (-1.017 to 1.702)	0.617
Total cholesterol(mmol/L)	0.095 (0.953)	0.009 (1.387)	0.086 (-0.491 to 0.662)	0.767
HDL(mmol/L)	0.037 (0.241)	0.038 (0.388)	-0.001 (-0.158 to 0.157)	0.994
Triglycerides (mmol/L)	-0.051 (0.753)	0.155 (2.746)	-0.205 (-1.206 to 0.796)	0.680
linoleic acid 9,11 (µmol/l)[1]	0.281 (5.709)	0.743 (6.046)	0.462 (-2.353 to 3.277)	0.744
linoleic acid 9,12 (µmol/l)[1]	-13.3 (242.6)	3.6 (238.4)	16.9 (-98.0 to 131.8)	0.770
linoleic acid 9,11:9,12(%)[1]	0.126 (0.537)	0.092 (0.614)	-0.034 (-0.311 to 0.243)	0.808
Calcium (mmol/L)	-0.021 (0.109)	-0.046 (0.093)	0.025 (-0.023 to 0.073)	0.299
Magnesium (mmol/L)	0.009 (0.092)	0.000 (0.090)	0.009 (-0.035 to 0.052)	0.683
Bilirubin(µmol/L)	-0.110 (5.924)	-0.150 (2.959)	0.043 (-2.165 to 2.252)	0.969
Alkaline phosphatase (µL)	22.8 (228.8)	17.2 (63.2)	5.6 (-73.5 to 84.7)	0.888
Total protein (g/L)	0.00 (6.616)	-1.70 (5.714)	1.70 (-1.24 to 4.64)	0.254
Albumin (g/L)	1.51 (4.513)	0.85 (3.429)	0.66 (-1.24 to 2.57)	0.487
CA 19-9 (mU/ml)	1.70 (15.772)	0.21 (9.343)	1.49 (-4.63 to 7.61)	0.628
CRP (g/L)	-7.27 (30.51)	-5.18 (28.01)	-2.09 (-16.05 to 11.87)	0.766

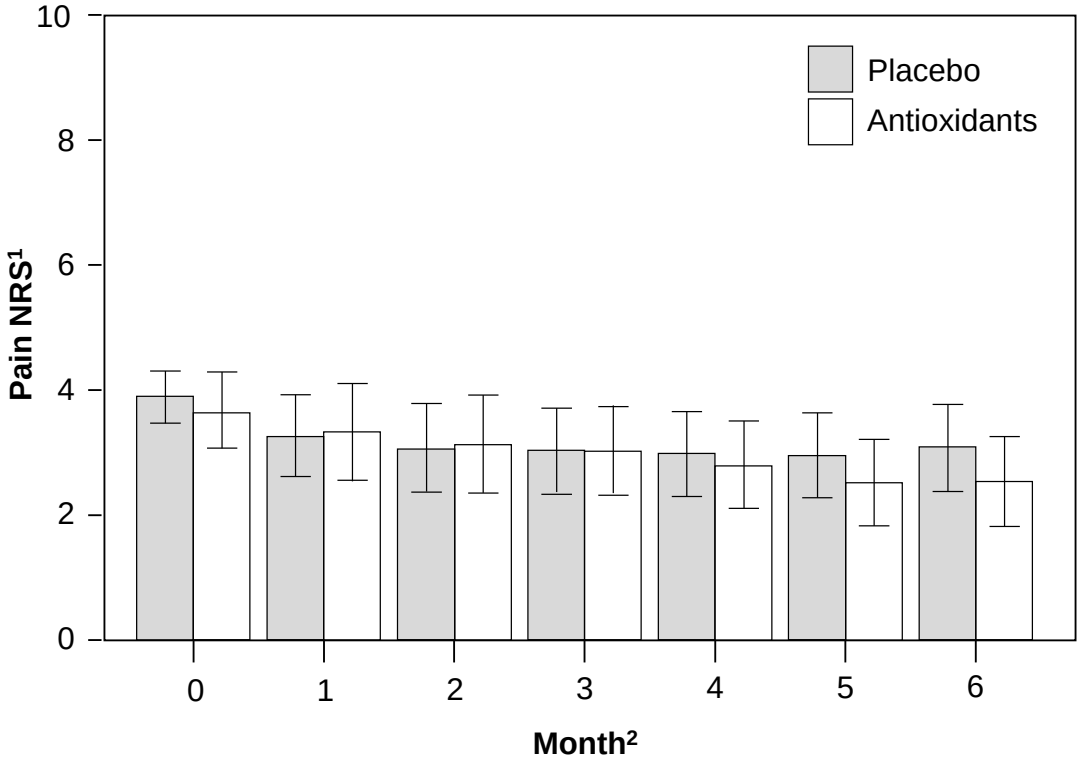
1 Unpaired t test on change score (6 month – baseline) values , **CRP**= C reactive protein, **HDL**= High density lipoprotein, **µL**=microlitre, **mmol**=milimol, **µmol**=micromol**, g**=gram, **WGSH**= Whole blood glutathione, **Rbc**=red blood cell, **Hb**=hemoglobin

Figure 2: Clinic NRS Pain Scores



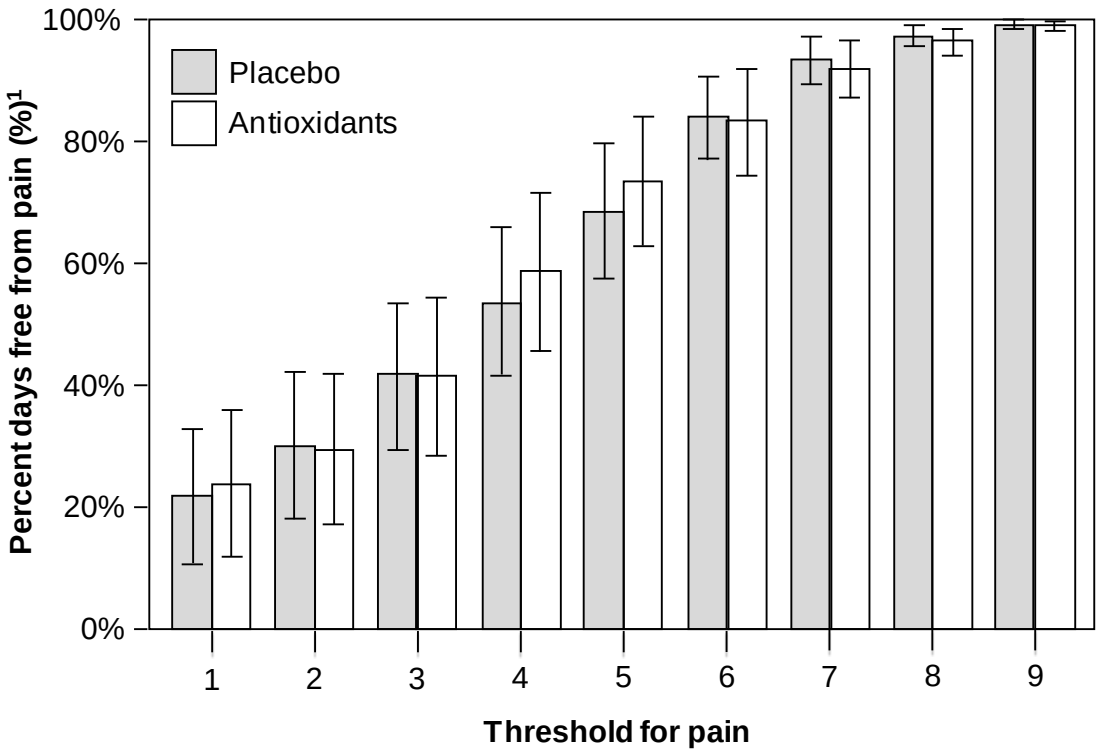
1 Mean pain score on the day of the clinic, on a numerical rating scale 0-10 (bars show 95%CI)

Figure 3: Diary NRS Pain Scores



1 Mean of daily diary pain scores in the previous month, on a numerical rating scale 0-10 (bars show 95%CI)
2 Monthly average of daily scores; month 0 denotes the month preceding trial treatment

Figure 4: Percentage of days free from pain by pain threshold



1 Percentage of days that pain scored below threshold (bars show 95%CI)