EDITORIAL

Micronutrient Therapy for Chronic Pancreatitis: Premises and Pitfalls

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The 'ANTICIPATE' trial professing the death knell for micronutrient antioxidant therapy in chronic pancreatitis was reported last September [1] and rubber-stamped by pundits [2, 3], but is flawed by misconception and wrong patient selection. In view of the six-month delay to publication of cautionary letters [4] and that misgivings were not allayed by the authors' riposte [5], it might be helpful to update the treatment's rationale in relation to nociception, and to indicate pitfalls in testing.

The characteristics of the disease are well known: 'acute pancreatitis' is the common harbinger, representing paralysis of apical exocytosis ('pancreastasis') in acinar cells; further episodes tend to follow; the propensity to intraductal calculi is associated with insufficiency of bicarbonate in pancreatic juice; fibrosis due to stellate cell activation may strangle sensory nerves, as also tubular structures in and around the gland; sooner or later pain predominates with threats of narcotic addiction, social upheaval and job loss; eventually all secretory parenchyma is obliterated, leading to maldigestion and diabetes [6].

The sensitization of pancreatic nociceptors within a milieu of sustained inflammation is now regarded as the critical initiating event in pain genesis, the afferent barrage leading to sensitization of higher-order neurons and thence central sensitization. The molecular agents are established: nerve growth factor (NGF); transforming growth factor beta (TGF-beta), which is also a potent activator of stellate cells; NGF-responsive gene products including the transient receptor potential vallinoid 1 (TRPV1), substance P (SP) and calcitonin gene-related peptide (CGRP); and the crucial mediator of central pain, brain-derived neurotrophic factor (BDNF) [7, 8, 9, 10]. As time goes by this pervading neurogenic assault renders insignificant the contribution to pain from dwindling flares of pancreatitis or compromised flow through tubular structures.

Key words Antioxidants; Mast Cells; Nociceptors

Abbreviations BDNF: brain-derived neurotrophic factor; CGRP: calcitonin gene-related peptide; CYP: cytochrome P450; CYP2E1: ethanol-inducible form of cytochrome P450; CYP1A: polycyclic aromatic hydrocarbon-inducible form of cytochrome P450; GSH: glutathione in its bioactive form; NGF: nerve growth factor; PAR-2: proteinase-activated receptor 2; ROS reactive oxygen species; RXS: reactive xenobiotic species; SP: substance P; TGF-beta: transforming growth factor beta; TRPV1: transient receptor potential vallinoid 1

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The corollary is that peripheral sensitization must be prevented by speedy removal of the primary pro-inflammatory factors. That is precisely what micronutrient therapy strives to achieve in correcting electrophilic stress, which has been identified as: i) disease detonator by causing pancreastasis; and ii) its inflammatory motor when reactive metabolites are shunted into the interstitium and unleash mast cells [6, 11]. Studies in transgenic mice have debunked the notion that recurrent trypsin activation in acinar cells underlies the disease [12]. Furthermore, there is no extracellular trypsin until well into an attack [13], when autoactivation seems to be involved [14].

The excess load of electron transfer reactions ('free radical activity') relative to extinguishing potential ('antioxidants') - which defines electrophilic stress - likely originates during xenobiotic processing via induced cytochrome P450 mono-oxygenases (CYP) [6, 11, 15]. Reactive oxygen species (ROS) are invariably generated thereby, but the evidence incriminates reactive xenobiotic species (RXS) derived from volatile petrochemicals in the occupational and/or domestic environment - whether in 'alcoholic', 'tropical', or 'idiopathic' disease [6, 16]. A small dose of ethanol induces CYP, so augmenting tissue damage by RXS from

petrochemical solvents and also from dibutyltin - an occupational chemical which yields a good animal model [6]. Chronic ethanol consumption leads to its processing by CYP2E1 with tissue damage from excess ROS alone, as could be germane to hereditary pancreatitis [11]. Inhaled smoke constituents - co-factor for the disease - strongly induce CYP1A, yield a range of RXS, and injure the gland [6]. Also of note in relation to autoimmune pancreatitis, ROS and CYP-derived RXS are implicated in other autoimmune diseases [17].

RXS, including from opiates, and linoleic acid-oxidation products elicit an anaphylactoid (non-immunoglobulin E) reaction of mast cells [13] with discharge of histamine, hydrolases (tryptase, chymase, phospholipase A_2, plasmin), numerous cytokines, and nociceptive factors. It has long been realized that histamine evokes an axon reflex with release of SP [13], but the mast cell-nociception link now goes much further. Mast cells synthesise, store and release NGF [18] which protects indirectly against RXS [19]. They express TRPV1 [20], which responds to stressors including hydrostatic pressure [21]. Tryptase, which unlike trypsin is resistant to alpha-1 proteinase inhibitor [13], awakens proteinase-activated receptor 2 (PAR-2) on the basolateral membrane of acinar cells, which might help to overcome the secretory blockade [22]: unfortunately PAR-2 also increases the expression and release of BDNF in microglia [23]. Mast cells co-secrete TGF-beta1 and its chymase activator [24]. A vicious cycle is set in motion because SP and NGF reactivate the cells [25]. These findings explain why mast cell control is proposed for treatment of complex regional pain syndromes [26].

This approach is not of itself applicable to chronic pancreatitis, however, as shown by the inefficacy of curcumin in a clinical trial [27] - although it is both an antioxidant [11], and inhibits the anaphylactoid response of mast cells [28]. Haem oxygenase shares these attributes [29] and is already recruited, as revealed by large amounts of bilirubin in patients' bile [13]. The inference is that these substances do not protect the critical intra-acinar target of oxidant attack.

That target (the methionine trans-sulphuration pathway) was pinpointed by 1960 through prescient investigations using halogenated hydrocarbons or a choline-deficient ethionine-supplemented diet. The pathway yields methyl and thiol (mainly glutathione, GSH) moieties that are indispensable for apical exocytosis; GSH protects essential enzymes in the route; ascorbic acid (bioactive form of vitamin C) has a reciprocal interaction with GSH; selenium enables GSH recycling; and the thiol-ascorbate axis secures the cystic fibrosis transmembrane conductance regulator - allowing it to facilitate exocytosis, and the passage into pancreatic juice of mucin-solubilizing bicarbonate and GSH [6, 11].

Dietary studies in CYP-induced patients with idiopathic chronic pancreatitis, and comparison of symptom change upon treatment with different micronutrient preparations highlighted the combination of methionine and ascorbic acid as a potentially successful prescription wherewith to rectify the on-going interrelated factors of methionine malutilization, pancreastasis and pancreatic electrophilic stress [6, 11]: clinicians are familiar with the use of methionine or another GSH precursor, N-acetylcysteine, to treat RXS-hepatotoxicity from paracetamol. Moreover: GSH, ascorbic acid, selenium and vitamin E stabilize mast cells [30, 31] and reduce fibrosis [11]; N-acetylcysteine blocks NGF-induced hydrogen peroxide signaling [32]; and an ascorbic acid analogue ameliorates in dietary and dibutyltin pancreatitis [6, 11]. Of course, micronutrients serve vital roles, over-and-above as antioxidants. The same is true for GSH - not least, as a potent inhibitor of trypsin [22].

Thus, logically, a prescription of micronutrients should enable first-line therapy for painful chronic pancreatitis. The dividend should be highest if treatment begins early, when its efficacy could be monitored by a fall in attack frequency. Lowered background pain, less analgesic usage, and improved life quality would gauge success when treatment starts after peripheral sensitization is under way. Little gain can be anticipated, however, after the bulk of secretory parenchyma is eradicated, in that free radical activity falls *parri passu* [33]. Wide variation in pain pattern and intensity suggest that a switch-over trial is preferable to a parallel design, but needs a long wash-out period at crossover because today's high-potency material has a substantial carry-over effect [34], in contrast to that used in the 1990 pilot assessment [35] - which included patients with recurrent attacks (5 of 23) that can presage chronic pancreatitis.

The success of micronutrient therapy has been validated by seven trials [6, 36] and two long-term accounts [6]. Four trials were placebo-controlled, of which the latest recorded concurrent lowering of a fibrosis marker in blood [36]. The good outcome was independent of geography, putative aetiology and predisposing gene mutation(s). The studies have been reviewed many times over [2, 11, 15, 37, 38].

The 'ANTICPATE' trial is the exception [1] - surprising because the authors had earlier documented improved quality of life, lowered pain scores and less analgesic usage in patients who were already on micronutrient therapy compared to values in an otherwise matched group on standard treatment [39]. The turnabout reflects breach of the central tenet of treatment, namely, electrophilic stress at baseline: there was none. The authors' [1, 5] and commentators' assertion [2, 3] that treatment failure was shown by unchanged pain and life-quality indices despite huge micronutrient increments in blood - to highly toxic values [4], but probably incorrect and due to careless reporting - exposes misapprehension. It is tantamount to pronouncing penicillin ineffective against bacterial infection when fever is due to, say, collagen vascular disease. In other words the trial actually compared placebo with a cosmetic substance - a fatal fissure that cannot be plastered over by a seducing plethora of life-quality assessments. The argument that a preponderance of alcoholics (51 of 70) accounted for the negative result is thus unwarranted - and in any case ignored results from Brno (59 of 70) [33] and misquoted data from Dehli (40 of 127) [40].

The misconception enfolds another, namely, that low micronutrient status equates to electrophilic stress - but all it denotes is susceptibility when and if the load of electron transfer reactions increases precipitously and/or is sustained at a higher-than-normal level. Electron spin resonance and chemiluminescense measurements show that this occurs in advance of any other anomaly within acinar cells during experimental pancreatitis [11, 13], but these techniques are not generally applicable in clinical studies. Instead, investigators seek 'fingerprints' of persisting stress. The choice from the immense library must be guided by the perceived target of attack - whether lipid, protein or DNA. There is no perfect 'marker', but when the threat is foremost on lipids, the best current marker is F_2 isoprostane, and 'thiobarbituric acid reacting substances' the least specific [41].

The triggering attack in pancreatitis is on enzymes and receptors that are protected by ascorbic acid interacting with GSH [11]. Hence, informatory measures in plasma/serum might include the percent oxidized ascorbic acid relative to total vitamin C [16]; GSH coupled with gamma-glutamyl transpeptidase activity [16]; protein carbonyls [42]; and allantoin, which signifies oxidation of uric acid: tests of 'total antioxidant activity' in blood reflect mainly uric acid and other bulk antioxidants [43], and are misleading in assessing the impact of micronutrient therapy [34]. If a nomogram is available, so much the better - as when the now outmoded marker, percent molar ratio of 9,11 to 9,12 linoleic acid is examined alongside selenium concentration [35]. Since dysregulated methionine metabolism due to RXS seems to underlie chronic pancreatitis, an index of its repair by treatment would be most helpful - as by analyzing a metabolite(s) [35, 44], and/or by ^{11}C methionine isotope scanning [45]: these resources are scarce, but an increase in erythrocyte GSH upon micronutrient therapy [40] is an indirect pointer.

Electrophilic stress is consistently recorded in patients with active chronic pancreatitis [11, 15]. Hence, the flip-side of the negative 'ANTICIPATE' trial in 70 patients is that pain in most patients was not of pancreatic origin - flouting the second treatment proviso. i) The diagnosis was uncertain in nine who had normal or minimal-changes on endoscopic pancreatography but no secretory study [6]. ii) Faecal elastase less than 100 µg/g showed severe exocrine insufficiency (Pancreatic Elastase 1™ Stool Test, Schebo® - Biotech AG, Giessen, Germany) in several. Gut pain due to undigested fat [46] - whether or not accompanied by steatorrhoea [47] - is a major contributor to poor life quality. iii) Narcotic addiction, as shown by high opiate consumption per day, causes gut dysmotility [6], and also intractable pain that is indistinguishable from that of chronic pancreatitis [48]. iv) Readers are not told how many, if any, of the trial participants were in employment - salient, in that occupational chemicals are connected to disease development [6, 16]. That is why participants in the original trial were asked not to alter lifestyle for the duration [35]. v) It is also not disclosed how many of the 50% of patients with prior intervention had a duct-drainage procedure - which should blunt pancreatic electrophilic stress and mast cell-activated pain circuits [21], at least in the short term.

The damage to pancreatology wrought by this unsound but influential study cannot be overstated and could steer patients towards unnecessary intervention [49], even total pancreatectomy - resurrecting images of peptic ulcer disease pre-*H pylori*. Pancreatologists brave enough to mount future trials of micronutrient therapy should now be aware how not to go about it.

Conflict of interest The author has no potential conflict of interests

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