Commentary to accompany Lin et al 'Sodium benzoate, a D-amino acid oxidase inhibitor…'

D-amino acid oxidase (DAO) inhibition: a new glutamate twist for clozapine augmentation in schizophrenia?

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Despite clozapine, many patients with schizophrenia remain treatment-resistant. Unfortunately, after many clinical trials with a diverse range of agents, there remain no evidence-based clozapine augmentation strategies. Against this background, the positive results of the randomised, double-blind, placebo-controlled trial of adjunctive sodium benzoate reported by Lin et al (1; this issue) are extremely welcome, and complement their earlier trial showing similar benefits in patients taking other antipsychotics (2). Equally, history teaches us to interpret results in this field cautiously, and to consider carefully the evidence for efficacy as well as the hypothesized mechanism of action.

Lin et al (1) studied sixty Taiwanese patients with schizophrenia who had persistent symptoms despite being on clozapine. The patients were randomised to addition of sodium benzoate (1g/day or 2g/day) or placebo. The patients were more severely and chronically ill than in many comparable studies, and all were inpatients. The latter fact likely contributed to the remarkable 98% completion rate for the six-week trial. There were four primary outcomes: Positive and Negative Symptom Scale (PANSS) total score; Scale for the Assessment of Negative Symptoms (SANS); Global Assessment of Functioning (GAF); and Quality of Life Scale (QoL). Secondary outcomes included PANSS positive and negative symptom scores, Hamilton depression rating scale, and a range of cognitive measures.

Both doses of sodium benzoate were more effective than placebo on the SANS (effect size as measured by Cohen's d = 0.65 and 0.72). The higher dose was also effective for PANSS total score (d = 0.80), QoL (d = 0.82) and PANSS positive scale (d = 0.86). All other outcomes, including GAF and cognition, did not differ between groups. Sodium benzoate was well tolerated, with no extra-pyramidal side-effects and no impact on neutrophil count.

The authors conclude, justifiably, that the trial shows a beneficial adjunctive effect of sodium benzoate on positive and negative symptoms of schizophrenia in patients taking clozapine. Appropriately, they also note a number of caveats, of which two are most important. After correction for having four primary outcomes and five secondary outcomes, significant effects of sodium benzoate were limited to the 2mg/d dose, with improvements on the PANSS (total and positive scores) and QoL. Also, although the effect sizes are moderate to large, the improvement in terms of points on the rating scales is much less impressive (e.g. a 4 point reduction on PANSS total score compared to placebo), and hence of questionable clinical relevance. The modest extent of the benefits is emphasised by the lack of change in GAF, and the fact that negative symptoms as assessed by the PANSS negative scale did not change significantly despite the reduction on the SANS. On the other hand, any statistically robust improvement in this refractory group of patients is encouraging, and it is possible that greater benefits might accrue from longer treatments, larger doses, or if administered with concurrent psychosocial or pro-cognitive interventions.

Sodium benzoate is a food preservative and, at first sight, an unlikely treatment for schizophrenia. However, it was discovered many years ago to be an inhibitor of the enzyme D-amino acid oxidase (DAO, DAAO) and this forms the basis for its use, according to the following rationale (3). DAO metabolises the amino-acid D-serine, which is the major endogenous co-agonist of the synaptic N-methyl-D-aspartate (NMDA) subtype of ionotropic glutamate receptor (4). In schizophrenia, DAO expression and enzyme activity is increased, and D-serine levels may be decreased (3,5). Reducing DAO activity with sodium benzoate increases D-serine availability and thence enhances NMDA receptor function. Parenthetically, DAO was a prominent candidate gene for schizophrenia, but the locus has not been supported by genome-wide association studies.

Despite its attractiveness, each step in this hypothesized sequence of events linking sodium benzoate to NMDA receptor signalling is open to question. Establishing the potential value of sodium benzoate (or other DAO inhibitors) in schizophrenia will benefit from careful examination of these issues.

Firstly, it is not certain that sodium benzoate is acting via DAO inhibition. Although Lin et al (1) measured blood DAO levels, they did not measure its activity, and circulating D-serine levels did not change in response to either dose of sodium benzoate. Moreover, preclinical data are varied regarding the ability of oral sodium benzoate to impact DAO activity and D-serine levels in the brain. It therefore remains possible that sodium benzoate is acting independent of DAO.

Secondly, assuming inhibition of DAO activity is the basis for the therapeutic benefits, where is this occurring? Presumably this is in the brain, although the high levels of DAO in the kidney and some other peripheral tissues should be noted. Within the brain, DAO is conventionally considered to be limited to the cerebellum, to be expressed in astrocytes but not neurons, and to be localised to peroxisomes (organelles involved in beta-oxidation and other catabolic reactions); see Figure, panel A. None of these distributional properties would normally be associated with antipsychotic effects, and have led to some understandable scepticism about the potential value of DAO inhibition for schizophrenia. However, recent findings suggest a more ‘schizophrenia-relevant’ profile of DAO (Figure, panel B). Thus, DAO is in fact expressed in many brain regions, including hippocampus and frontal cortex (6), and midbrain, wherein it modulates dopamine release (7). The latter functionality is notable because, in contrast to its widespread expression in the brain, detectable DAO *activity* is extremely low outside the cerebellum – a dissociation which remains unexplained. At the cellular level, DAO mRNA and protein are found in neurons not just in glia; moreover, within neurons, DAO is localised to the presynaptic zone, much better placed to influence D-serine levels in the vicinity of synaptic NMDA receptors than it would be if DAO were present exclusively in glial peroxisomes.

Thirdly, what is the relevant DAO substrate? DAO metabolises other D-amino acids than just D-serine, including D-alanine, itself an NMDA receptor co-agonist. Inhibition of these other substrates might help explain why sodium benzoate appears to have greater therapeutic efficacy in schizophrenia than is observed with D-serine administration. Indeed, the efficacy of sodium benzoate seen by Lin et al (1) contrasts with the absent or even deleterious effects seen when augmenting clozapine with other NMDA receptor-enhancing strategies (e.g. addition of D-serine or glycine) (1).

Fourthly, are the benefits of DAO inhibition mediated solely by increasing NMDA receptor co-agonist availability? As examples of alternative possibilities, D-serine is also the agonist at the Gluδ2 (GluD2) receptor; and DAO inhibition may affect oxidative processes, as suggested by the fact that Lin et al (1) found an increase in catalase activity in the 2g/d sodium benzoate treatment group.

Adding to these complexities, there are species differences in some properties of DAO which hinder translation from preclinical pharmacological studies and the interpretation of findings in DAO knockout mice. A specific issue concerns the possible regulation of DAO by a gene called G72 (also known as DAO activator or DAOA), which is primate-specific and which itself shows some evidence of genetic association to schizophrenia (8).

Overall, whilst a positive NMDA receptor modulatory action remains the most likely explanation for the reported therapeutic effects of sodium benzoate, much remains to be determined, and additional mechanisms may exist. Having noted the many uncertainties, the positive findings of Lin et al (1) argue for larger, longer, and more mechanistic clinical trials of sodium benzoate in schizophrenia. The results also give impetus to further studies of the neurobiology and pharmacology of DAO, and to the development of DAO inhibitors of greater potency and selectivity than sodium benzoate (9), and which can avoid the potential toxicity associated with its long-term, high-dose usage (10).

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Figure. DAO, D-serine and the NMDA receptor.

Panel A: Conventional view. 1: D-serine (triangle) is released by astrocytes, and possibly by neurons, into the synaptic cleft where it binds to the synaptic NMDA receptor (NMDAR) and acts as the co-agonist to accompany the agonist, glutamate (circle). 2: D-serine is removed from the synapse into glia, where it is degraded by DAO localised within peroxisomes (ellipse). In this simple model, sodium benzoate is presumed to act therapeutically by inhibiting astrocytic DAO, leading to increased D-serine availability at the NMDA receptor. For clarity, the panel does not show the other major NMDA receptor co-agonist, glycine, which is regulated via separate pathways and mechanisms (and which itself has been targeted in schizophrenia).

Panel B: Additional pathways which may be relevant to the therapeutic efficacy of sodium benzoate in schizophrenia. 3: DAO is present in some neurons, and concentrated at synaptic terminals. 4: DAO metabolises a range of substrates, including D-alanine (square), which is also an NMDA receptor co-agonist. 5: D-serine has actions beyond simply being an NMDA receptor co-agonist. 6: Sodium benzoate has effects other than DAO inhibition. 7: DAO has roles in addition to D-amino acid metabolism (hexagon). Steps 3-7 are not mutually exclusive, nor comprehensive, and their importance in human brain *in vivo* is not well established. However, they illustrate some of the ways by which the therapeutic benefits of sodium benzoate reported by Lin et al (1) may be mediated. For additional discussion, see text and ref. 3.