TECHNICAL COMMENTARY

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Nitric oxide

Introduction

Nitric oxide (NO) is a gas that acts as a signalling molecule in the CNS. It mediates cellular communication via cyclic GMP second messenger systems, activating guanylate cyclase, and its actions influence neurotransmitter release, learning and memory systems, and it also plays a key role in neurodevelopment. NO is produced endogenously by the conversion of L-arginine into L-citruline by nitric oxide synthase (NOS) enzyme[1]. There are three NOS isoforms, inducible NOS (iNOS), which is released in response to pathogens, as well as endothelial (eNOS) and neuronal (nNOS), which are expressed constitutively. NO is a highly reactive free radical, and is rapidly converted into other forms. NO reacts with molecular oxygen and accumulates in the plasma as nitrate NO3- and nitrite NO2-, which can contribute to oxidative stress[1, 2]. Disturbances in NO formation or release could interfere with the known functions of NO activity, including neural maturation and synapse formation, which could have relevance for possible neurodevelopmental aetiology of schizophrenia.

Method

We have included only systematic reviews (systematic literature search, detailed methodology with inclusion/exclusion criteria) published in full text, in English, from the year 2000 that report results separately for people with a diagnosis of schizophrenia, schizoaffective disorder, schizophreniform disorder or first episode schizophrenia. Reviews were identified by searching the databases MEDLINE, EMBASE, CINAHL, Current Contents, PsycINFO and the Cochrane library. Hand searching reference lists of identified reviews was also conducted. When multiple copies of reviews were found, only the most recent version was included. Reviews with pooled data are prioritised for inclusion.

Review reporting assessment was guided by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist, which describes a preferred way to present a meta-analysis[3]. Reviews rated as having less than 50% of items checked have been excluded from the library. The PRISMA flow diagram is a suggested way of providing information about studies included and excluded with reasons for exclusion. Where no flow diagram has been presented by individual reviews, but identified studies have been described in the text, reviews have been checked for this item. Note that early reviews may have been guided by less stringent reporting checklists than the PRISMA, and that some reviews may have been limited by journal guidelines.

Evidence was graded using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group approach where high quality evidence such as that gained from randomised controlled trials (RCTs) may be downgraded to moderate or low if review and study quality is limited, if there is inconsistency in results, indirect comparisons, imprecise or sparse data and high probability of reporting bias. It may also be downgraded if risks associated with the intervention or other matter under review are high. Conversely, low quality evidence such as that gained from observational studies may be upgraded if effect sizes are large, there is a dose dependent response or if results are reasonably consistent, precise and direct with low associated risks (see end of table for an explanation of these terms)[4]. The resulting table represents an objective summary of the available evidence, although the conclusions are solely the opinion of staff of NeuRA (Neuroscience Research Australia).

We found three systematic reviews that met our inclusion criteria[1, 2, 5].

- Moderate quality evidence suggests a reduction in NO metabolites in first episode and untreated schizophrenia, which may reverse over time or with antipsychotic treatment.

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Ng F, Berk M, Dean O, Bush AI

Oxidative stress in psychiatric disorders: evidence base and therapeutic implications

International Journal of Neuropsychopharmacology 2008; 11: 851-876

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Comparison	**Comparison of markers of oxidative stress: oxidants and antioxidants, in people with schizophrenia vs. controls.**
Summary of evidence	**Moderate to low quality evidence (large sample, direct, no measure of precision or consistency) suggests NO is altered in people with schizophrenia compared to controls, but the direction of the change is unclear.**
NO	
9 observational studies, N = 384 (patients only) NO was elevated in post-mortem and plasma studies, reduced in CSF studies, and no difference reported in platelet studies. Authors conclude NO is altered in people with schizophrenia, but the direction of changes is unclear.	
Consistency in results‡	No measure of consistency is reported.
Precision in results§	No confidence intervals are reported.
Directness of results‖	Direct

Maia-de-Oliveira JP, Trzesniak C, Oliveira IR, Kempton MJ, de Rezende TMN, Iego S, Baker GB, Dursun SM, Machado-de-Sousa JP, Hallak JEC

Nitric oxide plasma/serum levels in patients with schizophrenia: a systematic review and meta-analysis

Revista Brasileira Psiquiatria 2012; 34(Suppl2): S149-S162

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Comparison	**Plasma or serum levels of nitric oxide (NO) in people with**

	schizophrenia vs. controls.
Summary of evidence	**Moderate quality evidence (large samples, direct, inconsistent, some imprecision) suggests increased total plasma/serum NO levels in medicated people with schizophrenia, with no differences between drug-free patients and controls. Longer duration of illness is related to increased NO levels.**
Total nitrite plasma/serum NO	
No significant difference was found between all patients and controls; 10 studies, N = 844, *g* = 0.285, 95%CI -0.205 to 0.774, *p* = 0.254, I^2 = 90.8%, *p* < 0.001 *Significant, medium-sized effect of increased plasma/serum total nitrite in medicated patients compared to controls;* 5 studies, N = 502, *g* = 0.663, 95%CI 0.365 to 0.961, *p* < 0.001, I^2 = 56.0%, *p* = 0.059 *No significant difference was found between drug-free patients and controls;* 5 studies, N = 342, *g* = -0.109, 95%CI -0.856 to 0.637, *p* = 0.774, I^2 = 90.7%, *p* < 0.001 Meta-regression analysis showed a significant moderating effect of mean duration of illness (7 studies; *r* = 0.153, *p* = 0.005) in the overall analysis and a trend effect in the drug-free analysis (4 studies; *r* = 0.147, *p* = 0.057), such that increased duration of illness predicted increased effect size. There were no significant moderators in the medicated analysis. There was no evidence of publication bias.	
Consistency in results	Inconsistent
Precision in results	Precise, apart from drug-free patients analysis.
Directness of results	Direct

Oliveira JP, Zuardi AW, Hallak JE

Role of nitric oxide in patients with schizophrenia - A systematic review of the literature

Current Psychiatry Reviews 2008; 4(4): 219-227

View review abstract online

Comparison	**Multi-disciplinary comparison of nitric oxide (NO) and its metabolic associates in people with schizophrenia vs. controls.**

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Summary of evidence	**Moderate to low quality evidence (large samples, direct, unable to assess precision or consistency) suggests a reduction in NO metabolites in first episode and untreated schizophrenia patients, which may reverse over time or with antipsychotic treatment.**
Biochemical investigations of NO levels	
13 observational studies investigated NO metabolite levels, primarily measured through blood concentrations, one study measured NO levels in CSF; 6/13 studies (N = 600) found increased NO metabolite levels in people with schizophrenia. This sample primarily included chronic, treated people. 7/13 studies (N = 500) found decreased NO metabolite levels in people with schizophrenia. This sample primarily included first episode, drug naive, untreated, and deficit-subtype schizophrenia.	
Histochemical investigations of NO levels	
14 observational studies investigated NO metabolite levels, measured post-mortem; Basal ganglia: 3 studies (N = 92) One study found increased nitrite and nitrate levels in the caudate. Two studies found decreased NADPH-positive interneurons (modulating NO-mediated neurotransmission) in the basal ganglia. Cerebellum: 3 studies (N = 83) Two studies found increased NADPH-positive interneurons (modulating NO-mediated neurotransmission) and NOS-immunoreactive Purkinje cells in the cerebellum. One study found no difference in NOS-immunoreactive Purkinje cells in the cerebellum. Hypothalamus: 2 studies (N = 46) One study found reduced NOS-immunoreactive neurons in the paraventricular nucleus. One study found no difference in NO metabolite levels. Prefrontal cortex (PFC) / Temporal cortex: 6 studies (N = 129) 3 studies found reduced NO metabolite levels in PFC. 1 study found increased neuronal NOS mRNA levels in the PFC. 2 studies found decreased NO metabolites in the temporal neocortex but increases in the deeper temporal white matter.	
Genetic investigations of NO levels	

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9 observational studies investigated NO haplotypes associated with schizophrenia; *5 studies investigated neuronal NOS codifying gene, N = 3270;* 4/5 studies found nNOS gene alterations (SNPs) comprising several haplotypes, which were associated with schizophrenia. *4 studies investigated the CAPON gene, N = 2934;* 3/4 studies found CAPON gene SNPs comprising several haplotypes, which were associated with schizophrenia.
Pharmacological investigations of NO levels
4 observational studies investigated the effect of pharmacological substances on NO metabolite levels; Three studies reported on the effects of antipsychotic treatment alone on NO metabolite levels in schizophrenia with inconsistent results: one reported no group differences; the second reported increased NO levels prior to treatment which were reduced following olanzapine, which also correlated to PANSS scores; and the third reported reduced NO levels prior to treatment which were increased following risperidone (in untreated patients). One study reported on the effects on methylene blue adjuvant treatment, and reported increased CGI scores.

Consistency in results	No measures of consistency is reported.
Precision in results	No confidence intervals are reported.
Directness of results	Direct

Explanation of acronyms

CAPON = Carboxyl-terminal PDZ-ligand of neuronal NOS gene, CGI = Clinical Global Impressions, CSF = cerebrospinal fluid, mRNA = messenger ribonucleic acid, N = number of participants, NADPH = Nicotinamide adenine dinucleotide phosphate, NO = nitric oxide, NOS = nitric oxide synthase, nNOS = neuronal NOS, *p* = statistical probability of obtaining that result ($p < 0.05$ generally regarded as significant), PANSS = Positive and Negative Symptoms Scale, PFC = Prefrontal cortex, SNP = Single Nucleotide Polymorphism, vs = versus

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Explanation of technical terms

* Bias has the potential to affect reviews of both RCT and observational studies. Forms of bias include; reporting bias – selective reporting of results; publication bias - trials that are not formally published tend to show less effect than published trials, further if there are statistically significant differences between groups in a trial, these trial results tend to get published before those of trials without significant differences; language bias – only including English language reports; funding bias - source of funding for the primary research with selective reporting of results within primary studies; outcome variable selection bias; database bias - including reports from some databases and not others; citation bias - preferential citation of authors. Trials can also be subject to bias when evaluators are not blind to treatment condition and selection bias of participants if trial samples are small[6].

† Different effect measures are reported by different reviews.

Weighted mean difference scores refer to mean differences between treatment and comparison groups after treatment (or occasionally pre to post treatment) and in a randomided trial there is an assumption that both groups are comparable on this measure prior to treatment. Standardided mean differences are divided by the pooled standard deviation (or the standard deviation of one group when groups are homogenous) which allows results from different scales to be combined and compared. Each study's mean difference is then given a weighting depending on the size of the sample and the variability in the data. 0.2 represents a small effect, 0.5 a medium effect, and 0.8 and over represents a large treatment effect[6].

Reliability and validity refers to how accurate the instrument is. Sensitivity is the proportion of actual positives that are correctly identified (100% sensitivity = correct identification of all actual positives) and specificity is the proportion of negatives that are correctly identified (100% specificity = not identifying anyone as positive if they are truly not).

Odds ratio (OR) or relative risk (RR) refers to the probability of a reduction (< 1) or an increase (> 1) in a particular outcome in a treatment group, or a group exposed to a risk factor, relative to the comparison group. For example, a RR of 0.75 translates to a reduction in risk of an outcome of 25% relative to those not receiving the treatment or not exposed to the risk factor. Conversely, an RR of 1.25 translates to an increased risk of 25% relative to those not receiving treatment or not having been exposed to a risk factor. An RR or OR of 1.00 means there is no difference between groups. A mediumeffect is considered if RR > 2 or < 0.5 and a large effect if RR > 5 or < 0.2[7]. lnOR stands for logarithmic OR where a lnOR of 0 shows no difference between groups. Hazard ratios measure the effect of an explanatory variable on the hazard or risk of an event.

Correlation coefficients (eg r) indicate the strength of association or relationship between variables. They are an indication of prediction, but do not confirm causality due to possible and often unforseen confounding variables. An r of 0.10 represents a weak association, 0.25 a medium association and 0.40 and over represents a strong association. Unstandardised (*b*) regression coefficients indicate the average change in the dependent variable associated with a 1 unit change in the dependent variable, statistically controlling for the other

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independent variables. Standardised regression coefficients represent the change being in units of standard deviations to allow comparison across different scales.

Prevalence refers to how many existing cases there are at a particular point in time. Incidence refers to how many new cases there are per population in a specified time period. Incidence is usually reported as the number of new cases per 100,000 people per year. Alternatively some studies present the number of new cases that have accumulated over several years against a person-years denominator. This denominator is the sum of individual units of time that the persons in the population are at risk of becoming a case. It takes into account the size of the underlying population sample and its age structure over the duration of observation.

‡ Inconsistency refers to differing estimates of treatment effect across studies (i.e. heterogeneity or variability in results) that is not explained by subgroup analyses and therefore reduces confidence in the effect estimate. I^2 is the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error (chance) - 0% to 40%: heterogeneity might not be important, 30% to 60%: may represent moderate heterogeneity, 50% to 90%: may represent substantial heterogeneity and 75% to 100%: considerable heterogeneity. I^2 can be calculated from Q (chi-square) for the test of heterogeneity with the following formula;

$$I^2 = \left(\frac{Q - df}{Q}\right) \times 100\%$$

§ Imprecision refers to wide confidence intervals indicating a lack of confidence in the effect estimate. Based on GRADE recommendations, a result for continuous data (standardised mean differences, not weighted mean differences) is considered imprecise if the upper or lower confidence limit crosses an effect size of 0.5 in either direction, and for binary and correlation data, an effect size of 0.25. GRADE also recommends downgrading the evidence when sample size is smaller than 300 (for binary data) and 400 (for continuous data), although for some topics, this criteria should be relaxed[8].

‖ Indirectness of comparison occurs when a comparison of intervention A versus B is not available but A was compared with C and B was compared with C, which allows indirect comparisons of the magnitude of effect of A versus B. Indirectness of population, comparator and or outcome can also occur when the available evidence regarding a particular population, intervention, comparator, or outcome is not available so is inferred from available evidence. These inferred treatment effect sizes are of lower quality than those gained from head-to-head comparisons of A and B.

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