Antipsychotic Medication and Oxidative Cell Stress: A Systematic Review

Peter Lepping, MD, MRCPsych, MSc; John Delieu, PhD; Richard Mellor, MB, ChB, MRCPsych; John H. H. Williams, PhD; Peter R. Hudson, PhD; and Claire Hunter-Lavin, PhD

Objective: To look at (1) the association between antipsychotics and cell stress, (2) whether first-generation antipsychotics may show different effects than second-generation antipsychotics, and (3) whether recommendations can be made regarding medication.

Data Sources: We conducted a systematic review of 5 databases for all articles published until December 31, 2007: PubMed, Ovid MEDLINE, EMBASE, PsycINFO, and EBM Reviews. Under specific headings (eg, "heat shock proteins" and "oxidative stress"), a systematic search of these databases included such terms as *HSP70* and *homocysteine*, and specific search strings were constructed. No limits were placed on the year or language of publication. References from pertinent articles or books were retrieved.

Study Selection: We included 42 articles of human studies from 2,387 references originally retrieved. We included only articles that (1) were quantitative; (2) referred only to human tissue, in vivo, or in vitro; (3) stated what tissue was examined; (4) identified what metabolites were measured; and (5) had references.

Data Extraction: All articles were assessed by 2 authors, which ensured that the inclusion criteria were met. The selected studies were too heterogeneous to be combined for any useful meta-analysis. Three authors, therefore, independently interpreted the data, using specified criteria to judge whether each study showed a beneficial, detrimental, or no effect on the markers measured.

Data Synthesis: The analysis revealed no conclusive association with direct or indirect markers of oxidative cell stress and antipsychotics. For every reviewed antipsychotic, we revealed differing research results showing a beneficial, detrimental, or no effect. This was true for in vivo as well as in vitro studies.

Conclusions: It remains unclear whether antipsychotics increase or reduce cell stress. Claims of neuroprotective properties of antipsychotics seem premature.

J Clin Psychiatry 2011;72(3):273–285

Submitted: *April 7, 2009; accepted September 16, 2009.*
Online ahead of print: *June 29, 2010 (doi:10.4088/JCP.09r05268yel).*
Corresponding author: *Peter Lepping, MD, Wrexham Academic Unit, Croesnewydd Rd, Wrexham, LL13 7TY, Wales, UK (peter.lepping@wales.nhs.uk).*

The use of antipsychotics in patients with schizophrenia is widespread and recommended.[1] Furthermore, antipsychotic medication has recently been claimed to be neuroprotective,[2–4] and its early use in first-onset psychosis has been advocated widely.[5] In a recent major review Ng et al[6] found consistent, robust, and multidimensional evidence that oxidative cell stress is involved in the pathogenesis of schizophrenia and bipolar affective disorder, with weaker evidence for its involvement in other mental disorders. They point out that "evidence for the interdependent relationships between oxidative pathways and those involving neurotransmitters, hormones and inflammatory mediators further enhance the plausibility of the oxidative stress hypothesis, and provide a unifying framework for the various conceptual theories of causality."[6(p867)] We fully acknowledge that the etiology of schizophrenia is multifactorial, but Ng and colleagues'[6] evidence points to oxidative stress as an important factor in the biologic aspects of etiology and mortality in patients with schizophrenia. We therefore decided to conduct a systematic review to look at (1) whether there is any evidence that antipsychotic medication is associated with or causes cell stress or has beneficial effects on cell stress, (2) whether first-generation antipsychotics show different effects than second-generation antipsychotics (including clozapine), and (3) whether any 1 medication can be shown to be particularly beneficial with regard to cell stress.

Background

Long-term use of antipsychotic medication is associated with a number of adverse effects, such as neutropenia and agranulocytosis,[7,8] hyperprolactinemia,[9] cardiomyopathy,[10,11] weight gain,[12,13] and metabolic changes,[14] which include insulin resistance and hypertension. Antipsychotic medication is also associated with an increased risk of developing type II diabetes, which exacerbates the already elevated risk of cardiovascular problems and diabetes in people who use antipsychotics long term.[15] Although the development of neuroleptic malignant syndrome is associated with the use of all antipsychotic medication, it more commonly occurs with first-generation antipsychotics than with second-generation antipsychotics.[16]

The finding that oxidative stress is implicated in the etiology of major mental illness raises the possibility that oxidative cell stress arising from the bioactivation of antipsychotic drugs may impose an additional oxidative burden upon tissues that are already stressed and could result in cell death through apoptosis or necrosis. This process has been implicated in the pathogenesis of the metabolic syndrome.[17] Furthermore, oxidative stress has been associated with agranulocytosis through the formation of chemically reactive nitrenium iron-containing metabolites[11] and free radical formation.[18] It is also known that people with schizophrenia who are on antipsychotic medication have less mature neutrophils compared to healthy unmedicated volunteers,[7,8] implying an immunologic effect. This effect seems least significant for sulpiride, flupentixol, and fluphenazine but is highly statistically significant for olanzapine, risperidone, haloperidol, thioridazine, and trifluoperazine. In the case of olanzapine, the finding was slightly surprising given that it was previously considered to have little association with

Table 1. Systematic Review Search Terms—Heat Shock Proteins (HSPs), Oxidative Stress, and Antipsychotic Medication

Heading	Search Terms	Examples of Search Strings
HSP	*Stress proteins*, *HSP70*, *HSP72*, *HSP32*, *Haem oxygenase*, and *HO-1*	Antipsychotic OR benperidol OR olanzapine OR clozapine OR amisulpride OR aripiprazole OR chlorpromazine OR flupenthixol OR flupentixol OR haloperidol OR quetiapine OR risperidone OR sertindole OR thioridazine OR trifluoperazine OR zotepine OR fluphenazine OR levomepromazine OR methotrimeprazine OR pericyazine OR periciazine OR perphenazine OR pimozide OR prochlorperazine OR promazine OR sulpiride OR zuclophenthixol OR aripiprazole OR zotepine OR pipotiazine
Oxidative stress	*Oxidative stress*, *oxidative damage*, *hyperoxic*, *hyperoxia*, *oxidant*, *pro-oxidant*, *antioxidant*, *oxygen radicals*, *free radicals*, *superoxide*, *singlet oxygen*, *nitric oxide*, *nitric oxide toxicity*, *hydrogen peroxide*, *homocysteine*, *cell death*, *necrosis*, and *apoptosis*	(Oxidative adj stress) OR (Oxidative adj damage) OR TBARS OR 542-78-9 OR 29343-52-0 OR 107-02-8 OR (F2 adj isoprostane) OR (glutathionylated adj protein) OR (carbonylated adj protein) OR 3604-79-3 OR 7298-90-0 OR 54788-30-6 OR 980-21-2 OR 88847-89-6
Antipsychotic medication	*Olanzapine*, *clozapine*, *risperidone*, *benperidol*, *amisulpride*, *aripiprazole*, *chlorpromazine*, *flupenthixol*, *flupentixol*, *haloperidol*, *quetiapine*, *risperidone*, *sertindole*, *thioridazine*, *trifluoperazine*, *zotepine*, *fluphenazine*, *levomepromazine*, *methotrimeprazine*, *pericyazine*, *periciazine*, *perphenazine*, *pimozide*, *prochlorperazine*, *promazine*, *sulpiride*, *zuclophenthixol*, *aripiprazole*, *zotepine*, and *pipotiazine*	(superoxide adj dismutase) OR (glutathione adj peroxidize) OR catalase OR 70-18-8 OR 27025-41-8 OR 1406-18-4 OR 11103-57-4 OR 50-81-7 OR TAC OR TRAP OR 454-28-4 OR Hsp70 OR Hsp72 OR (heme adj oxygenase) OR hyperoxic OR hyperoxia OR oxidants

Abbreviations: adj = adjacent, TAC = total antioxidant capacity, TBARS = thiobarbituric acid reactive substances, TRAP = total radical-trapping antioxidant parameter.

agranulocytosis.[19] All this is accumulative evidence that oxidative stress is a possible mechanism for antipsychotic side effects.

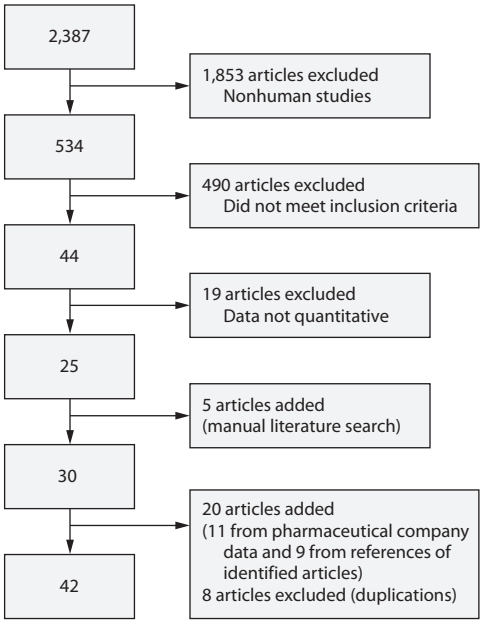
Oxidative stress may also have an effect on vascular endothelium.[20] Equally affected by oxidative cell stress are heat shock proteins (HSPs), which have a variety of protective intracellular duties and show increased expression in response to stress to protect cells from stress-induced damage.[21] Heat shock proteins are known to be affected by peripheral vascular disease[22] and cardiovascular disease,[23] and oxidative stress has been suggested as a possible reason for this.[23] Nothing is known about HSP levels in the context of antipsychotic medication usage. HSP70 is induced by many physiologic stresses including oxidative stress,[21] and HSP32 is induced specifically by oxidative stress.[24] While HSPs are traditionally regarded as intracellular proteins, it is now known that they are present extracellularly and are correlated to certain disease processes[22,25,26] and in vitro cellular stress.[27–30] Serum HSPs may be of use in the monitoring of disease should they be shown to be affected by antipsychotic drugs. Heat shock protein release from cells in culture is another method for monitoring stress induced by antipsychotics in in vitro studies. It is interesting to consider that schizophrenic patients are at increased risk of premature death due to cardiovascular disease, with a relative risk of 1.5 of patients dying from cardiovascular disease.[31] If oxidative stress were a side effect of antipsychotic medication, it could help explain this worrying increase in cardiovascular disease.

DATA SOURCES AND EXTRACTION

We conducted a systematic review of 5 databases for all articles published until December 31, 2007—PubMed (produced 1,568 results), Ovid MEDLINE (produced 868 results), EMBASE (produced 1,008 results), PsycINFO (produced 88 results), EBM Reviews (produced 41 results). Under specific headings, eg, "heat shock proteins" and "oxidative stress," a systematic search of these databases included such terms as *HSP70* and *homocysteine* (Table 1), and specific search strings were constructed. No limits were placed on the year or language of publication. References from pertinent articles or books were retrieved using the following criteria: (1) only articles that were quantitative; (2) those that referred only to human tissue, in vivo, or in vitro; (3) articles that stated what tissue was examined; (4) articles that identified what metabolite/metabolites were measured; and (5) those that had references. We looked for homogeneity within the identified studies. Our aim was to pool any homogenous studies for a meta-analysis. In the case of heterogeneity, we planned to look at each substance individually. We pooled all the markers we were looking at into 3 categories:

- Category 1 combined all markers of direct oxidative stress, including malondialdehyde/thiobarbituric acid reactive substances, reactive oxygen species/reactive oxygen metabolites, hydrogen peroxide (H_2O_2), and O_2. These are all a direct measure of oxidants (eg, H_2O_2) or the result of oxidative activity (eg, malondialdehyde).
- Category 2 consisted of indirect markers of oxidative stress, including superoxide dismutase, glutathione, glutathione peroxidase, catalase, essential polyunsaturated fatty acids, HSP antibodies and antioxidants. These are all induced by oxidative stress and are typically part of the cellular defense against oxidative stress.
- Category 3 consisted of other markers of immune response such as proinflammatory, anti-inflammatory, or markers of neutrophil shift or danger. These are also induced by oxidative stress, but are not part of an antioxidant defense but directly relate to the immune response.

Figure 1. Data Selection Flowchart



We assessed all selected studies and extracted all available data on any antipsychotic mentioned or combined data on first- or second-generation antipsychotics. We looked at the available in vitro data on human cells separately from the in vivo data. The lead author and 2 of the coauthors (J.H.H.W., P.L., and P.R.H.) individually assessed whether the substance had a beneficial (positive) or a detrimental (negative) effect on cells. Generally, a rise in direct or indirect markers of cell stress was interpreted as negative while a reduction was interpreted as positive. Among the immune markers, a reduction of proinflammatory markers, a rise in anti-inflammatory markers, a reduction in the neutrophil shift, and a reduction in hemolysis and danger markers were seen as positive. The opposite was interpreted as a negative effect.

SELECTION OF ARTICLES

Two reviewers (J.D. and R.M.) independently searched the 5 databases, finding 2,387 abstracts after the removal of duplicates (Figure 1). We wrote to all pharmaceutical companies manufacturing antipsychotics, requesting information regarding published or unpublished trials or work in progress on these drugs and their associations with cell stress. A database of these abstracts was created within a reference management software package (EndNote, Thomson-Reuters, New York, New York). We then applied the terms *subject* or *patient* to our database to exclude nonhuman data (Figure 1). This process excluded 1,853 articles from the identified abstracts, giving us a total of 534 abstracts. Further processing excluded 490 articles as they did not meet the inclusion criteria. In this group some had no references, others were similar articles written by the same authors with slightly different titles but submitted to different journals. They were treated as duplicates. Another substantial number of articles looked at related subjects such as tardive dyskinesia rather than schizophrenia or did not record any cell response.

All articles were assessed by 2 reviewers, which ensured that the inclusion criteria were met. A further 19 articles that contained no measurable parameters were excluded, leaving 25. Five articles were identified by manual literature search, 11 were added from the pharmaceutical industry, and 9 from the references within the identified articles bringing, the total up to 50 articles. Then 8 articles were identified as duplications and excluded, leaving a total of 42 included articles.

The selected studies were too heterogeneous to be combined for any useful meta-analysis. Some failed to give details for individual antipsychotics but combined a selection of first-generation antipsychotics.[32–36] Some examined more than 1 cell stress marker.[35,37–40] Many studies looked at more than 1 effect and more than 1 antipsychotic, which explains why the total number of reported effects for a particular drug or effect does not necessarily correspond with the total number of studies showing that effect. One study[41] met the inclusion criteria but did not yield results at 2 different points in time and was therefore not included in the analysis. We show the referenced results for all individual antipsychotics in an interpreted format in Table 2. Table 3 shows a summary description of all included studies with their design, quality, and outcome. Table 4 shows the actual observed change for all studies and substances.

We excluded animal studies as they can be poor models for human cell behavior. Furthermore, we wanted to increase the relevance of the review to psychiatric practice by focusing on human cells.

DATA SYNTHESIS

First-Generation Antipsychotics

In summary, 11 studies[32,34,35,38,42–48] looked at different first-generation antipsychotics and direct oxidative stress markers. Most of them used haloperidol. Two of the studies[42,46] showed a beneficial effect; 5 studies,[35,43,44,49,50] a detrimental effect; and 6 studies,[32,34,38,42,45,48] no effect on cells. When we considered markers of indirect oxidative stress, 3 studies[40,51,52] showed a beneficial effect; 6 studies,[32,34,36,40,50,53] a detrimental effect; and 7 studies,[33,35,37,38,43,47,54] no significant effect. Regarding markers for immune responses, 1 study[55] showed a beneficial effect on inflammation and similar markers, 6 studies[8,49,50,56–58] showed a detrimental effect, and 2 studies[8,48] showed no effect. One of these studies was Delieu et al,[8] which showed that chlorpromazine, trifluoperazine, thioridazine, flupenthixol, and sulpiride caused a neutrophil shift to less mature cells, while no such effect was seen with fluphenazine.

Second-Generation Antipsychotics

For the second-generation antipsychotics, the results were equally ambivalent. When we looked at direct oxidative stress markers, 3 studies[59–61] showed a beneficial effect, 5 studies[18,35,62–64] showed a detrimental effect, and

Table 2. Summary of Results: Number of Studies Using an Antipsychotic That Resulted in a Positive, Negative, or No Effect on Cell Stress/Immune Response[a]

	Direct Markers			Indirect Markers			Immune Markers		
Antipsychotic	Positive Effect	Negative Effect	No Effect	Positive Effect	Negative Effect	No Effect	Positive Effect	Negative Effect	No Effect
SGA									
All SGAs	7	5	18	12	18	14	4	8	4
Clozapine	3	4	5	3	3	5	2	6	0
Risperidone	1	1	5	5	6	5	1	1	1
Olanzapine	1	0	4	2	7	3	1	1	1
Amisulpride	0	0	2				0	0	1
Quetiapine	1	0	2	1	1	0	0	0	1
Ziprasidone	1	0	0	1	1	1			
FGA									
All FGAs	2	9	12	6	10	24	1	11	2
FGA (combined)	0	1	2	0	3	3			
Haloperidol	1	4	3	4	2	6	1	4	1
Chlorpromazine	1	1	2	0	1	5	0	3	0
Flupenthixol	0	0	1				0	1	0
Fluphenazine	0	0	1	0	0	4	0	0	1
Thioridazine	0	0	1	0	0	2	0	1	0
Trifluoperazine				2	0	0	0	1	0
Sulpiride	0	0	1	0	0	2	0	1	0
Zuclopenthixol	0	0	1	0	0	2			
Bromperidol				0	2	0			
Molindone				0	2	0			
Prochlorperazine	0	1	0						
Metoclopramide	0	1	0						
Levomepromazine (methotrimeprazine)	0	1	0						

[a]This table describes positive, negative, and no effect on cells. The total for FGAs and SGAs sums up all individual results from all studies included. Some studies examined multiple substances or used more than 1 marker. Generally, a rise in direct or indirect markers of cell stress was interpreted as negative, while a reduction was interpreted as positive. Among the immune markers, a reduction of proinflammatory markers, a rise in anti-inflammatory markers, a reduction in the neutrophil shift, and a reduction in hemolysis and danger markers were seen as positive.
Abbreviations: FGA = first-generation antipsychotic, SGA = second-generation antipsychotic.

7 studies[32,34,38,43,45,48,63] showed no significant effect. The 1 prospective randomized controlled study[45] we identified showed a tendency toward a positive effect for clozapine, risperidone, and amisulpride but a tendency toward a negative effect for olanzapine compared to first-generation antipsychotics. However, when we did our own *t* tests using mean and standard deviations comparing day 21 with day 0 for all drugs, none of them were significant. Even those comparisons that appeared to be the most promising at face value between first-generation antipsychotics and olanzapine had a *P* value of $P = .1$ in both cases. Regarding markers of indirect cell stress, 5 studies[40,52,59,65,66] showed a beneficial effect, 5 studies[2,32,34,39,62] showed a detrimental effect, and 4 studies[33,35,38,43,53] showed no significant effect. Looking at markers of inflammation, we found 2 studies[55,67] that showed a beneficial effect (ie, reduced inflammatory markers), 7 studies[8,49,57,58,68–70] that showed a detrimental effect, and 1 study[48] that showed no effect. A neutrophil shift toward less mature cells was found with risperidone and olanzapine.[8]

In Vitro Studies

To summarize, no substance had exclusively beneficial or detrimental results across the board. First-generation antipsychotics do not come out better or worse than second-generation antipsychotics. With regard to the consequences of taking antipsychotics on cell stress and immune response markers, no definite conclusion can be drawn about potential harm or benefit from antipsychotic medication.

DISCUSSION

Given the recent independent research on second-generation antipsychotics and their relative risk for metabolic disturbance and weight gain as well as efficacy compared to first-generation antipsychotics, it is paramount that we gain more knowledge about potential risks and benefits of antipsychotic medication. Any suggestion from the data regarding beneficial effects on cell stress would have been highly welcomed. However, our results are inconclusive for every substance analyzed, with almost as many studies showing beneficial effects across the board as studies showing detrimental effects. We therefore cannot draw any conclusion as to whether antipsychotic medication increases the risk of cell stress, nor can we make any specific recommendation of 1 substance to be less likely to increase cell stress than another. It is not possible to conclude that antipsychotic medication does increase cell stress either, and no such assumption of increased metabolic risk should be drawn from our results.

There is now increasingly unambiguous evidence that oxidative stress is involved in the etiology of schizophrenia and bipolar affective disorder, probably also in major depression and other mental disorders.[6] This evidence in itself demands further research in order to explore whether this new knowledge may lead to future treatment options. These include antioxidants and related substances,[71] some of which have already been successfully tested in the treatment

Table 3. List of Included Studies[a]

Study	N	Design	Cell Stress Marker	Antipsychotic(s) Used	Comments
Abdalla et al[37] (1986)	70 (58 controls)	Assess SOD and H_2O_2 levels in schizophrenic (n = 50) and bipolar (n = 20) patients	SOD, GSH-Px	Chlorpromazine, haloperidol, fluphenazine	SOD levels in treated *and* untreated schizophrenic patients were 60% higher than in healthy controls (40% higher in treated bipolar patients). No details were given for individual drugs. No drug group effect on SOD levels was found
Akyol et al[38] (2002)	100 (51 controls)	Between groups analysis of differences on oxidative stress markers in patients on neuroleptic medication and normal controls	Nitric oxide, TBARS, SOD, GSH-Px, xandine oxidase	Haloperidol, chlorpromazine, thioridazine, fluphenazine, olanzapine, sulpiride, risperidone, zuclopenthixol	There was increased xandine oxidase and nitric oxide, with decreased SOD and unchanged GSH in patients compared to controls. TBARS were increased in schizophrenic patients. The findings suggest that the changes in oxidative stress markers were due to the disease itself rather than the medication
Arolt et al[49] (2000)	29 (29 controls)	Longitudinal study of interferon-γ, interleukin-2, and soluble interleukin-2R levels over 28 days in treated patients and controls	Interferon-γ, interleukin-2, soluble interleukin-2R	Clozapine, chlorpromazine	Interferon-γ and interleukin-2 were lower in patients than in controls throughout the study. This, plus the correlation between interferon-γ and interleukin-2, suggests that the cytokine system of patients with schizophrenia resembles that of patients with an autoimmune disorder
Arvindakshan et al[59] (2003)	52 (45 controls)	Red cell membrane EPUFA and plasma TBARS were measured in never-medicated (n = 20) and medicated (n = 32) schizophrenia patients and in control subjects (n = 45)	Plasma TBARS, red cell membrane EPUFA	Clozapine, risperidone, olanzapine, FGAs	Reduced membrane EPUFA were found in never-medicated patients compared with control subjects. Lower levels of membrane EPUFA and higher levels of TBARS were associated with severe symptoms in never-medicated vs medicated patients
Atmaca et al[66] (2005)	29 (29 controls)	Randomized single-blind study of the effects of olanzapine with or without extract of Ginkgo biloba on measures of oxidative stress	SOD, GSH-Px, catalase	Olanzapine	Patients had higher baseline SOD, catalase, and GSH than controls. After treatment there was a greater decrease in catalase and SOD, but not GSH in the coadministration group, compared to the placebo group
Bindoli et al[42] (1987)	23	Assess MDA serum levels in schizophrenic patients treated with chlorpromazine at weeks 0 and 3; prospective study	MDA	Chlorpromazine	MDA levels were significantly reduced after 3 weeks of treatment with chlorpromazine
Bonelli et al[36] (2005)	50 (55 controls)	Comparison of transglutaminase in patients on antipsychotics and controls	Transglutaminase	FGA (melperone, flupentixol, haloperidol, prothipendyl) SGA (risperidone, zotepine, olanzapine)	FGAs and SGAs increased transglutaminase in cerebrospinal fluid, suggesting increased cerebral apoptotic cell death, especially in females
Dakhale et al[62] (2004)	48 (40 controls)	A prospective, open-label, 8-week study of the effects of SGAs on blood MDA, SOD, and the antioxidant ascorbic acid	MDA, SOD, ascorbic acid	Clozapine, risperidone, olanzapine, quetiapine, ziprasidone	SOD and MDA were higher in schizophrenic patients and ascorbic acid lower, compared to controls. Treatment with SGAs reduced MDA and SOD and increased plasma ascorbic acid
Delieu et al[8] (2006)	89 (58 controls)	Assessment of neutrophil maturity shift in patients taking antipsychotics	Neutrophil maturity	Flupenthixol, fluphenazine, haloperidol, thioridazine, trifluoperazine, sulpiride, olanzapine, risperidone	All antipsychotics tested except fluphenazine showed a shift toward more immature neutrophils after treatment with antipsychotics. Sulpiride just reached statistical significance, while olanzapine showed the biggest shift
Evans et al[39] (2003)	16 (25 controls)	Measure of EPUFA levels in first-episode psychotic patients treated with SGAs at weeks 0, 12, and 24; prospective study	EPUFA, SOD, GSH-Px, catalase	Risperidone, olanzapine	In patients, SOD levels were lower, catalase levels higher and GSH-Px levels unchanged at baseline. No change after 12 weeks. After 24 weeks, SOD levels were almost normalized, catalase levels had increased further. EPUFA levels were significantly increased in patients, no change after 12 weeks, slight reduction after 24 weeks. Data for risperidone and olanzapine combined

(continued)

Table 3 (continued). List of Included Studies[a]

Study	N	Design	Cell Stress Marker	Antipsychotic(s) Used	Comments
Fehsel et al[63] (2005)	28 (19 controls)	Assess effect of antipsychotics on SOD and proapoptotic gene expression	SOD	Clozapine, olanzapine, polymedication	Observation of dramatically increased numbers of native neutrophils stained for SOD production in all groups (and significantly elevated expression levels of proapoptotic genes)
Fischer et al[18] (1991)	NA	Assess role of free radical formation in clozapine-induced agranulocytosis; in vitro study	Free radicals	Clozapine	Human myeloperoxidase-oxidated clozapine. Radical adduct concentration was increased in presence of clozapine. Possibility of clozapine activation to free radical metabolites, causing oxidative stress
Gama et al[43] (2006)	17 (15 controls)	Measure serum SOD and TBARS in schizophrenic patients under treatment	TBARS, SOD	Haloperidol, clozapine	SOD and TBARS levels were significantly higher in patients than controls. TBARS were higher under clozapine than haloperidol; no such difference with SOD levels
Gross et al[60] (2003)	8	Assess ROS production in schizophrenic patients taking clozapine at week 3 and analyze relationship with EEG θ values; prospective study	ROS	Clozapine	On average, ROS increased significantly at week 3, although three-eighths of patients showed a reduction
Hinze-Selch et al[68] (1998)	17	Measure effect before and up to 6 weeks into clozapine treatment on immune parameters in vitro	Soluble interleukin-2R, interleukin-6	Clozapine	Clozapine suppressed soluble interleukin-2R and interleukin-6 independent of dose and body temperature. Serum immunoglobulin G levels were increased, autoantibody patterns unaffected
Hinze-Selch et al[69] (2000)	23	Randomized trial of the effects of clozapine therapy and clozapine and fluvoxamine cotherapy upon plasma cytokines	TNF-α, soluble TNF receptors p55 and p75, soluble interleukin-2R, leptin, blood granulocyte counts	Clozapine	The study examined the effects of the serotonin reuptake inhibitor fluvoxamine, which affects the hepatic metabolism of clozapine, on the immunomodulatory effects of clozapine. The coadministration of fluvoxamine attenuated the clozapine-induced increase in TNF-α, enhanced the clozapine-induced increase in leptin, and decreased granulocyte count
Jeding et al[44] (1995)	NA	In vitro study of ability of antipsychotics to show antioxidant or pro-oxidant action	Superoxide and hydroxyl radical scavenging	Chlorpromazine, haloperidol, prochlorperazine, metoclopramide, methotrimeprazine	No reaction with superoxide at a significant rate. Chlorpromazine was hydroxyl radical scavenger. Haloperidol showed no ability to inhibit lipid peroxidation or scavenge peroxyl radicals
Joffe et al[64] (1998)	8	ROS production in vitro by monocytes and polymorphonuclear leukocytes were measured before and after 3 and 10 weeks of clozapine treatment	ROS	Clozapine	Higher levels of plasma clozapine were associated with a decrease in ROS by monocytes, lower levels with an increase
Joffe et al[61] (1999)	8	Measure ROM production by in vitro clozapine-incubated blood phagocytes from 8 treatment-resistant schizophrenia patients newly started with clozapine at weeks 0, 3, and 10	ROS	Clozapine	ROM production decreased at weeks 3 and 10. Magnitude of decrease positively correlated with improvement of negative symptoms
Kamińska et al[50] (2003)	10 (30 controls)	Case report of a patient with hypersensitivity to neuroleptics: admitted to hospital and monitored for 8 days after discontinuation of treatment	Interleukin-1α, interleukin-6, TNF-α, ROS, catalase	Haloperidol	Serum levels of interleukin-1α, interleukin-6, and TNF-α changed significantly over the course of the observation period, forming waves with peak activity of interleukin-6 and TNF-α exceeding normal levels. ROS and catalase were also increased during this period
Khan et al[32] (2002)	52 (16 controls)	Measurement of differences between erythrocyte membrane EPUFA and plasma TBARS in drug-naive and long-term neuroleptic treatment	EPUFA (including AA and DHA), TBARS	FGAs (including haloperidol), clozapine, olanzapine, risperidone	EPUFA (especially AA and DHA) were lower in drug-naive patients. They also had higher TBARS. Levels of AA and DHA were lower and TBARS higher in chronically medicated patients than in controls. EPUFA levels were higher in chronically mediated patients than in drug-naive patients. Lower membrane AA and DHA probably predate the illness. Increased oxidative stress, either as part of the illness and/or as part of its treatment, may be responsible for the reduced EPUFA

(continued)

Table 3 (continued). List of Included Studies[a]

Study	N	Design	Cell Stress Marker	Antipsychotic(s) Used	Comments
Kim et al[40] (2001)	90 (83 controls)	Assess role of HSP in pathogenesis of schizophrenia by measuring antibodies to HSP; 70 patients were followed up for 6 weeks	Anti-HSP70, anti-HSP90	Clozapine, risperidone (46 patients); haloperidol, bromperidol, molindone, trifluoperazine (24 patients)	Schizophrenic patients had higher levels of antibodies against HSP70 and HSP90 than healthy controls. No difference of elevated HSP antibody levels between treated and drug-naive patients. Those followed up after 6 weeks of antipsychotic treatment showed decrease in HSP70 antibodies, but not in HSP90 antibodies. No details were given for individual drugs
Kim et al[55] (2002)	102 (85 controls)	Interleukin-12 levels were measured to assess the effect of antipsychotic medication in different major mental illnesses	Interleukin-12	Risperidone, olanzapine, clozapine, haloperidol, nemonapride	Interleukin-12 levels of patients with major depression were significantly higher than those with schizophrenia or bipolar affective disorder. After 8 weeks of antipsychotic treatment, interleukin-12 levels decreased significantly (irrespective of weight changes)
Kropp et al[45] (2005)	92	MDA levels were analyzed at days 0, 7, and 21 after starting an antipsychotic; prospective study	MDA	FGA (haloperidol or flupentixol), risperidone, olanzapine, clozapine, amisulpride, quetiapine	Most MDA levels were within normal limits. All SGAs had much lower MDA levels than FGA at days 0, 7, and 21, except olanzapine, whose MDA levels were no different to FGAs. Amisulpride and clozapine had the lowest MDA levels at day 21, followed by quetiapine and risperidone
Li et al[2] (1999)	NA	In vitro study assessing effect of olanzapine on SOD and nerve growth factor receptor p75	SOD, low-affinity nerve growth factor receptor p75	Olanzapine	Olanzapine increases SOD and decreases p75 gene expression. This has been associated with reduced cell death suggesting neuro-protective potential
Lieber et al[56] (1984)	NA	Assess interaction of chlorpromazine with human erythrocyte membrane in vitro	Human erythrocyte membrane	Chlorpromazine	Chlorpromazine caused hemolysis by a colloid-osmotic mechanism
Linday et al[41] (1995)	21 (26 controls)	Comparison of free-radical scavenging enzyme activity and related trace metal concentration between patients with clozapine agranulocytosis, patients with no agranulocytosis, and 2 groups of control subjects	GSH-Px in plasma and red cells, selenium	Clozapine	GSH in plasma was lowest in the clozapine-agranulocytosis group. Plasma selenium was lower in both clozapine-treated groups. The presence of at least 1 of lower plasma GSH, lower red cell GSH, or selenium distinguished the clozapine-agranulocytosis group from 1 of the control groups
Maes et al[67] (1994)	14 (26 controls)	A study of the effect of chronic treatment with clozapine upon cytokines and their soluble receptors in blood	Interleukin-6, soluble interleukin-6R, soluble interleukin-2R	Clozapine	Schizophrenia in younger patients is accompanied by increased interleukin-6 and soluble interleukin-2R secretion. Treatment with clozapine increased soluble interleukin-2R secretion
Neeman et al[33] (2005)	94 (34 controls)	Amino acid levels were compared in schizophrenic patients and healthy controls	MDA, homocysteine	Clozapine, risperidone, olanzapine, FGAs	Patients had significantly lower MDA and higher homocysteine levels; low MDA levels positively correlated with high level of negative symptoms. No separate analysis for different drugs
Pai et al[46] (1994)	15	Measure MDA and GSH levels in drug-naive psychotic patients' cerebrospinal fluid at weeks 0 and 2 after treatment with haloperidol; prospective study	GSH, MDA	Haloperidol	GSH cerebrospinal fluid levels decreased while MDA levels increased after haloperidol treatment. MDA increases were significant between 105% and 625%
Peet et al[47] (1995)	23 (16 controls)	Measure red blood cells membrane fatty acid composition and TBARS in treated schizophrenic patients	TBARS, EPUFA	Chlorpromazine equivalents	No difference in TBARS between schizophrenic and control groups. Those with low levels of arachidonic acid had significantly higher TBARS levels than those with high arachidonic acid levels. No correlation between medication dosage and TBARS levels
Pollmacher et al[70] (1996)	27	Assess effect of clozapine on immune system markers in schizophrenic patients at weeks 0, 2, and 6; prospective study	Interleukin-1 receptor antagonist, soluble interleukin-2 receptor, interleukin-6, TNFα, soluble TNF receptors p55 and p75	Clozapine	Clozapine increased the plasma levels of TNFα, soluble TNF receptors p55 and p75, and sIL-2R. No effect on plasma IL-1 receptor antagonist. Clozapine has consistent in vivo immunomodulatory effects. Clozapine-induced fever is mediated by pyrogenic cytokines

(continued)

Table 3 (continued). List of Included Studies[a]

Study	N	Design	Cell Stress Marker	Antipsychotic(s) Used	Comments
Ramchand et al[34] (1996)	11 (8 controls)	The susceptibility to nonenzymatic peroxidation by cumene hydroperoxide of erythrocyte membranes from patients taking neuroleptic treatment was measured in vitro	Cell membrane lipid peroxidation, indirectly measured	Chlorpromazine	Red cell membranes from treated schizophrenic patients were less susceptible to nonenzymatic oxidative damage than those from healthy controls. Pretreatment of cells from patients and controls with chlorpromazine showed further protection
Rudolf et al[57] (2002)	NA	In vitro study	Interleukin-2, interferon-γ	Haloperidol, clozapine, (amitriptyline)	Interleukin-2 and interferon-γ were enhanced by haloperidol and clozapine once stimulated with phytohemagglutinin, but not amitriptyline
Sarandol et al[48] (2007)	40 (35 controls)	Assess changes in blood levels of MDA, SOD, and S100B at days 0 and 42 after treatment with antipsychotics in schizophrenic patients; prospective study	MDA, SOD (S100B: marker for neurodegeneration)	Clozapine, olanzapine, amisulpride, risperidone (tablet and intramuscular depot), quetiapine, haloperidol	MDA, SOD, and S100B levels were significantly higher in schizophrenia group. Antipsychotics did not change MDA or SOD in treated schizophrenic group. Controls remained untreated. No results available for specific drugs; results were pooled
Skoblenick et al[53] (2006)		Comparison of apoptosis-inducing factor in striata of patients taking haloperidol or clozapine in comparison to controls	Aptosis-inducing factor	Haloperidol, clozapine	Haloperidol but not clozapine increased apoptosis-inducing factor in SH-SY5Y cells. Striatal sections from patients who had taken haloperidol also had increased nuclear apoptosis-inducing factor immunofluorescence signaling
Szuster-Ciesielska et al[58] (2004)	16	Measure the stimulated and unstimulated effect of in vitro cytokine production (inflammatory response) in healthy subjects	Interleukin-10, TGF-β, interleukin-12, interleukin-4, interlukin-2, ROS (O_2^-), H_2O_2, interferon-γ	Clozapine, chlorpromazine, haloperidol	All 3 antipsychotics significantly increased stimulated production of anti-inflammatory cytokines (interleukin-10, TGF-β) and unstimulated production of interleukin-10. No influence on interleukin-12 unstimulated, but inhibited stimulated interleukin-12 production. Haloperidol and chlorpromazine, but not clozapine, inhibited interleukin-4 and interferon-γ production
Yao et al[51] (1998)	38 (40 controls)	Within-subject, repeated measures on-off haloperidol study	SOD, GSH-Px, catalase in erythrocytes	Haloperidol	During drug-free conditions, SOD activities, but not GSH and catalase activities, were higher than controls. Within subjects, SOD and GSH, but not catalase, were higher in drug-free conditions. There was no correlation between plasma haloperidol levels and activity of any of the enzyme levels
Zhang et al[35] (2006)	92 (50 controls)	Measure antioxidant levels in medicated schizophrenic patients (paranoid, residual or disorganized subgroups)	SOD, GSH-Px, catalase, MDA	Clozapine, risperidone, FGA (not further specified)	SOD and GSH-Px levels were decreased in schizophrenia, MDA levels were elevated. Higher MDA, but lower catalase levels were noted in female compared to male patients. No differences between drugs
Zhang et al[65] (2003)	41 (40 controls)	Comparison of SOD levels in controls and patients over a 12-week period of risperidone treatment	SOD	Risperidone	Risperidone treatment significantly decreased the initially high blood SOD level in schizophrenia. This was accompanied by a diminishment of symptoms
Zhang et al[52] (2006)	78 (30 controls)	Double-blind randomized prospective study. SOD and prolactin levels measured at weeks 0 (after 2-week placebo lead-in) and 12 in chronic schizophrenic patients taking risperidone (n = 41) or haloperidol (n = 37)	SOD (prolactin)	Risperidone, haloperidol	Patients had significantly higher SOD levels at baseline than healthy controls. Risperidone- and haloperidol-treated patients both had a significant and similar reduction of SOD levels after 12 weeks of treatment. Prolactin levels increased more in risperidone than in haloperidol group at week 12
Zhang et al[54] (2001)	82 (30 controls)	Assess effect of haloperidol with and without Ginkgo biloba on SOD in chronic schizophrenic patients	SOD	Haloperidol	SOD levels were higher in schizophrenic patients than in healthy controls. After 12 weeks, the haloperidol + Ginkgo biloba group showed a significant reduction in SOD levels; the haloperidol + placebo group did not, but showed a tendency toward reduction

[a]All controls were healthy controls.

Abbreviations: AA = arachidonic acid, DHA = docosahexaenoic acid, EPUFA = essential polyunsaturated fatty acids, FGA = first-generation antipsychotic, GSH = glutathione, GSH-Px = glutathione peroxidase, H_2O_2 = hydrogen peroxide, HSP = heat shock protein, MDA = malondialdehyde, NA = not applicable, O_2^- = superoxide free radical, ROM = reactive oxygen metabolites, ROS = reactive oxygen species, SGA = second-generation antipsychotic, SOD = superoxide dismutase, TBARS = thiobarbituric acid reactive substances, TGF-β = transforming growth factor-β, TNF = tumor necrosis factor.

Table 4. Summary of Cell Responses

			Direct Oxidative Stress					Indirect Oxidative Stress								Immune				Cell Damage	
Study	Cell Stress Marker(s)	Antipsychotic	MDA/TBARS	ROS/ROM	H_2O_2	O_2-	GSH	SOD	GSH-Px	Catalase	EPUFA	Homocysteine	Antioxidant	HSP90 Antibodies	HSP70 Antibodies	Proinflammatory	Antiinflammatory	Immature Neutrophils	Danger	Hemolysis	Apoptosis
Abdalla et al[37] (1986)	SOD, GSH-Px	Chlorpromazine						—	—												
		Haloperidol						—	—												
		Fluphenazine						—	—												
Akyol et al[38] (2002)	Nitric oxide, TBARS, SOD, GSH-Px, XO	Haloperidol	—					—	—												
		Zuclopenthixol	—					—	—												
		Chlorpromazine	—					—	—												
		Thioridazine	—					—	—												
		Fluphenazine	—					—	—												
		Olanzapine	—					—	—												
		Sulpiride	—					—	—												
		Risperidone	—					—	—												
Arolt et al[49] (2000)	IFN-γ, IL-2, sIL-2R	Clozapine														+↑					
		Chlorpromazine														+↑					
Arvindakshan et al[59] (2003)	Plasma TBARS, red cell membrane EPUFA	Clozapine	—								+↑										
		Risperidone	—								+↑										
		Olanzapine	—								+↑										
		FGAs	—								+↑										
Atmaca et al[66] (2005)	SOD, GSH-Px, catalase	Olanzapine							+↓												
Bindoli et al[42] (1987)	MDA	Chlorpromazine	+↓																		
Bonelli et al[36] (2005)	Transglutaminase	FGAs (including haloperidol), SGAs (including olanzapine)																			+↑
Dakhale et al[62] (2004)	MDA, SOD, ascorbic acid	Clozapine	+↓					+↓					+↑								
		Risperidone	+↓					+↓					+↑								
		Olanzapine	+↓					+↓					+↑								
		Quetiapine	+↓					+↓					+↑								
		Ziprasidone	+↓					+↓					+↑								
Delieu et al[8] (2006)	Neutrophil maturity	Flupenthixol																+↑			
		Fluphenazine																—			
		Haloperidol																+↑			
		Thioridazine																+↑			
		Trifluoperazine																+↑			
		Sulpiride																+↑			
		Olanzapine																+↑			
		Risperidone																+↑			
Evans et al[39] (2003)	EPUFA, SOD, GSH, catalase	Risperidone						+↑		+↑	+↑										
		Olanzapine						+↑		+↑	+↑										
Fehsel et al[63] (2005)	SOD	Clozapine				+↑															
		Olanzapine				—															
Fischer et al[18] (1991)	Free radical generation from clozapine, ie, potential to produce ROS	Clozapine		+↑																	
Gama et al[43] (2006)	TBARS, SOD	Haloperidol	+↑					—													
		Clozapine	—					—													
Gross et al[60] (2003)	ROS	Clozapine		+↓																	

(continued)

© Copyright 2010 Physicians Postgraduate Press, Inc.

Table 4 (continued). Summary of Cell Responses

			Direct Oxidative Stress					Indirect Oxidative Stress								Immune				Cell Damage	
Study	Cell Stress Marker(s)	Antipsychotic	MDA/TBARS	ROS/ROM	H_2O_2	O_2-	GSH	SOD	GSH-Px	Catalase	EPUFA	Homocysteine	Antioxidant	HSP90 Antibodies	HSP70 Antibodies	Proinflammatory	Antiinflammatory	Immature Neutrophils	Danger	Hemolysis	Apoptosis
Hinze-Selch et al[68] (1998)	IL-6, sIL-2R	Clozapine														+↑					
Hinze-Selch et al[69] (2000)	TNF-α, soluble TNF receptors p55 and p75, sIL-2R, leptin, blood granulocyte counts	Clozapine														+↑					
Jeding et al[44] (1995)	OH· scavenging, inhibition of iron-dependent OH· generation, inhibition of lipid peroxidation, peroxyl radical scavenging, effect on heme-protein dependent lipid peroxidation, reaction with superoxide, scavenging of hypochlorous acid	Haloperidol		+↑																	
		Chlorpromazine											+↑								
		Prochlorperazine											+↑								
		Metoclopramide											+↑								
		Methotrimeprazine											+↑								
Joffe et al[64] (1998)	ROS	Clozapine		+↑																	
Joffe et al[61] (1999)	ROS	Clozapine		+↓																	
Kamińska et al[50] (2003)	IL-1α, IL-6, TNF-α, ROS, catalase	Haloperidol		+↑						+↑						+↑					
Khan et al[32] (2002)	EPUFA (including AA and DHA), TBARS	FGAs (including haloperidol)	—								+↑										
		Clozapine	—								+↑										
		Olanzapine	—								+↑										
		Risperidone	—								+↑										
Kim et al[40] (2001)	Anti-HSP70, anti-HSP90	Clozapine												+↓	+↓						
		Risperidone												+↓	+↓						
		Haloperidol												+↓	+↓						
		Bromperidol												+↓	+↓						
		Molindone												+↓	+↓						
		Trifluoperazine												+↓	+↓						
Kim et al[55] (2002)	IL-12	Risperidone															+↑				
		Olanzapine															+↑				
		Nemonapride															+↑				
		Clozapine															+↑				
		Haloperidol															+↑				
Kropp et al[45] (2005)	MDA	Haloperidol	—																		
		Flupenthixol	—																		
		Risperidone	—																		
		Olanzapine	—																		
		Clozapine	—																		
		Amisulpride	—																		
		Quetiapine	—																		
Li et al[2] (1999)	SOD, low-affinity nerve growth factor receptor p75	Olanzapine						+↑													
Lieber et al[56] (1984)	Human erythrocyte membrane	Chlorpromazine																		+↑	
Linday et al[41] (1995)	GSH-Px in plasma and red cells, selenium	Clozapine																			
Maes et al[67] (1994)	IL-6, sIL-6r, sIL-2R	Clozapine															+↑				
Neeman et al[33] (2005)	MDA, homocysteine	Clozapine										—									
		Risperidone										—									
		Olanzapine										—									
		FGAs										—									
Pai et al[46] (1994)	GSH, MDA	Haloperidol	+↑				+↓														
Peet et al[47] (1995)	TBARS, EPUFA	Chlorpromazine	—								—										

(continued)

© Copyright 2010 Physicians Postgraduate Press, Inc.

Table 4 (continued). Summary of Cell Responses

			Direct Oxidative Stress					Indirect Oxidative Stress								Immune				Cell Damage	
Study	Cell Stress Marker(s)	Antipsychotic	MDA/TBARS	ROS/ROM	H_2O_2	O_2-	GSH	SOD	GSH-Px	Catalase	EPUFA	Homocysteine	Antioxidant	HSP90 Antibodies	HSP70 Antibodies	Proinflammatory	Antiinflammatory	Immature Neutrophils	Danger	Hemolysis	Apoptosis
Pollmacher et al[70] (1996)	IL-1, IL-6, sIL-2R, TNF-α, soluble TNF receptors p55 and p75	Clozapine														+↑					
Ramchand et al[34] (1996)	Cell membrane lipid peroxidation, indirectly measured	Chlorpromazine											+↑								
Rudolf et al[57] (2002)	IL-2, IFN-γ	Haloperidol														+↑					
		Clozapine														+↑					
		Amitriptyline														—					
Sarandol et al[48] (2007)	MDA, SOD (S100B: marker for neurodegeneration—also sometimes called an "alarmin" or "danger signal")	Risperidone			—														—		
		Olanzapine			—														—		
		Clozapine			—														—		
		Quetiapine			—														—		
		Amisulpride			—														—		
		Haloperidol			—														—		
Skoblenick et al[53] (2006)	Apoptosis-inducing factor	Haloperidol																			+↑
		Clozapine																			—
Szuster-Ciesielska et al[58] (2004)	IL-2, IL-4, IL-10, IL-12, IFN-γ, TGF-β, ROS (O_2^-), H_2O_2	Clozapine				+↓											+↑				
		Chlorpromazine				+↓											+↑				
Yao et al[51] (1998)	SOD, GSH-Px and catalase in erythrocytes	Haloperidol							+↓												
Zhang et al[35] (2006)	SOD, GSH-Px, catalase, MDA	Clozapine	+↑					—	—												
		Risperidone	+↑					—	—												
		FGA (not specified)	+↑					—	—												
Zhang et al[65] (2003)	SOD	Risperidone						+↓													
Zhang et al[52] (2006)	SOD (prolactin)	Risperidone						+↓													
		Haloperidol						+↓													
Zhang et al[54] (2001)	SOD	Haloperidol						—													

Abbreviations: AA = arachidonic acid, DHA = docosahexaenoic acid, EPUFA = essential polyunsaturated fatty acids, FGA = first-generation antipsychotic, GSH = glutathione, GSH-Px = glutathione peroxidase, H_2O_2 = hydrogen peroxide, HSP = heat shock protein, IFN-γ = interferon-γ, IL = interleukin, MDA = malondialdehyde, O_2^- = superoxide free radical, OH· = hydroxyl radical, ROM = reactive oxygen metabolites, ROS = reactive oxygen species, SGA = second-generation antipsychotic, sIL = soluble interleukin, SOD = superoxide dismutase, TBARS = thiobarbituric acid reactive substances, TGF-β = transforming growth factor-β, TNF = tumor necrosis factor, XO = xanthine oxidase.
Symbols: + = an effect, — = no effect, ↑ = an increase in the variable, ↓ = a decrease in the variable.

of schizophrenia. Another important clinical implication is for the claims of neuroprotective properties attributed particularly to expensive second-generation antipsychotics. This has led to calls for the early use of antipsychotic medication in newly diagnosed schizophrenic patients.[5] Heat shock proteins are undoubtedly involved in diverse neuroprotective mechanisms,[72] making a link between oxidative stress and neuroprotection very plausible. Our findings suggest that claims of neuroprotective properties of antipsychotics may well be premature given that we obtained very inconclusive results from the current literature. Unless it were proven that oxidative stress and neuroprotective properties of antipsychotic medication are unrelated, which is highly unlikely given the current breadth of evidence, any definite claims of neuroprotection should be treated with caution. This may also mean that we need to rethink neuroprotection as a potential benefit in the argument for the early use of second-generation antipsychotics in newly diagnosed schizophrenic patients. One in vitro study[57] showed that haloperidol and clozapine cause a significant proinflammatory response in an activated system, indicating potential problems with inflammatory responses for patients on antipsychotic medication at times of infection or increased stress. This leaves open the possibility of varying responses according the current level of system activation.

Limitations of our study include the impossibility of making clear quality judgments about each study, as they do not fit into the usual quality assessment framework for randomized controlled trials. However, all included studies have a good or reasonable design and methodology for what

© Copyright 2010 Physicians Postgraduate Press, Inc.

they are intended to measure. Although they were all trials with human participants, the measured changes were usually in regard to blood markers and antipsychotics rather than changes in the participants themselves. The heterogeneity of the studies did not allow us to cluster any specific study designs, which rendered any comment on bias impossible.

There is a clear need for more prospective studies on humans in order to determine the risk on cells, including those involved in immune responses, that antipsychotic medication poses as well as any beneficial effects they may have. However, prior to this there is clearly a need for in vitro experiments using suitable cell culture systems looking at the potential cellular stress that may be induced by these compounds. There is also a case for the development and use of new, sensitive markers of cell stress to understand the metabolic effects of antipsychotic medication more fully. Such markers may include urine isoprostane[73] and markers of protein glycation, oxidation, and nitration.[74]

Clinically, the importance of this study is that claims of neuroprotective properties of antipsychotic medication seem premature given the inconclusiveness of our results.

Drug names: aripiprazole (Abilify), clozapine (FazaClo, Clozaril, and others), haloperidol (Haldol and others), metoclopramide (Metozolv, Reglan, and others), molindone (Moban), olanzapine (Zyprexa), pimozide (Orap), prochlorperazine (Compro, Procomp, and others), quetiapine (Seroquel), risperidone (Risperdal, Risperdal Consta, and others), ziprasidone (Geodon).

Author affiliations: Glyndŵr University, Wrexham (Dr Lepping), and Departments of Psychiatry (Drs Lepping and Mellor) and Clinical Biochemistry (Dr Hudson), Betsi Cadwaladr University Health Board; School of Medical Sciences, Bangor University (Dr Delieu), Bangor, North Wales; and Chester Centre for Stress Research (Dr Williams) and Chester Centre for Stress Research (Dr Hunter-Lavin), University of Chester, Chester, England, United Kingdom. Dr Hunter-Lavin is no longer with Chester Centre for Stress Research.

Potential conflicts of interest: None reported.
Funding/support: None reported.

REFERENCES

1. National Institute for Clinical Excellence. *Schizophrenia: Core Interventions in the Treatment and Management of Schizophrenia in Primary and Secondary Care*. London, United Kingdom: National Institute for Clinical Excellence; 2002.
2. Li XM, Chlan-Fourney J, Juorio AV, et al. Differential effects of olanzapine on the gene expression of superoxide dismutase and the low affinity nerve growth factor receptor. *J Neurosci Res*. 1999;56(1):72–75.
3. Wang H, Xu H, Dyck LE, et al. Olanzapine and quetiapine protect PC12 cells from beta-amyloid peptide(25–35)-induced oxidative stress and the ensuing apoptosis. *J Neurosci Res*. 2005;81(4):572–580.
4. Xu H, Wang H, Zhuang L, et al. Demonstration of an anti-oxidative stress mechanism of quetiapine: implications for the treatment of Alzheimer's disease. *FEBS J*. 2008;275(14):3718–3728.
5. Lieberman JA. Neuroprotection: a new strategy in the treatment of schizophrenia. Neurobiological basis of neurodegeneration and neuroprotection. *CNS Spectr*. 2007;12(suppl 18):4–6.
6. Ng F, Berk M, Dean O, et al. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *Int J Neuropsychopharmacol*. 2008;11(6):851–876.
7. Delieu JM, Badawoud M, Williams MA, et al. Antipsychotic drugs result in the formation of immature neutrophil leucocytes in schizophrenic patients. *J Psychopharmacol*. 2001;15(3):191–194.
8. Delieu JM, Horobin RW, Duguid JK. Formation of immature neutrophil leucocytes in schizophrenic patients treated with various antipsychotic drugs: comparisons and predictions. *J Psychopharmacol*. 2006;20(6):824–828.
9. Schyve PM, Smithline F, Meltzer HY. Neuroleptic-induced prolactin level elevation and breast cancer: an emerging clinical issue. *Arch Gen Psychiatry*. 1978;35(11):1291–1301.
10. Brakoulias V, Bannan E, Cohen P, et al. Amisulpride and cardiomyopathy. *Aust N Z J Psychiatry*. 2005;39(8):738.
11. Williams DP, O'Donnell CJ, Maggs JL, et al. Bioactivation of clozapine by murine cardiac tissue in vivo and in vitro. *Chem Res Toxicol*. 2003;16(10):1359–1364.
12. Hägg S, Spigset O. Antipsychotic-induced venous thromboembolism: a review of the evidence. *CNS Drugs*. 2002;16(11):765–776.
13. Haupt DW, Newcomer JW. Hyperglycemia and antipsychotic medications. *J Clin Psychiatry*. 2001;62(suppl 27):15–26, discussion 40–41.
14. Newcomer JW. Antipsychotic medications: metabolic and cardiovascular risk. *J Clin Psychiatry*. 2007;68(suppl 4):8–13.
15. Casey DE. Metabolic issues and cardiovascular disease in patients with psychiatric disorders. *Am J Med*. 2005;118(suppl 2):15S–22S.
16. Lepping P, Mukandan J, Poole R. Neuroleptic malignant syndrome and catatonia. *Prog Neurol Psychiatry*. 2007;11:9–14.
17. Dandona P, Aljada A, Chaudhuri A, et al. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. *Circulation*. 2005;111(11):1448–1454.
18. Fischer V, Haar JA, Greiner L, et al. Possible role of free radical formation in clozapine (clozaril)-induced agranulocytosis. *Mol Pharmacol*. 1991;40(5):846–853.
19. Bhana N, Foster RH, Olney R, et al. Olanzapine: an updated review of its use in the management of schizophrenia. *Drugs*. 2001;61(1):111–161.
20. Jiang F, Drummond GR, Dusting GJ. Suppression of oxidative stress in the endothelium and vascular wall. *Endothelium*. 2004;11(2):79–88.
21. Williams JH, Ireland HE. Sensing danger—Hsp72 and HMGB1 as candidate signals. *J Leukoc Biol*. 2008;83(3):489–492.
22. Wright BH, Corton JM, El-Nahas AM, et al. Elevated levels of circulating heat shock protein 70 (Hsp70) in peripheral and renal vascular disease. *Heart Vessels*. 2000;15(1):18–22.
23. Xu Q, Schett G, Li C, et al. Mechanical stress-induced heat shock protein 70 expression in vascular smooth muscle cells is regulated by Rac and Ras small G proteins but not mitogen-activated protein kinases. *Circ Res*. 2000;86(11):1122–1128.
24. Tsiftsoglou AS, Tsamadou AI, Papadopoulou LC. Heme as key regulator of major mammalian cellular functions: molecular, cellular, and pharmacological aspects. *Pharmacol Ther*. 2006;111(2):327–345.
25. Hunter-Lavin C, Hudson PR, Mukherjee S, et al. Folate supplementation reduces serum hsp70 levels in patients with type 2 diabetes. *Cell Stress Chaperones*. 2004;9(4):344–349.
26. Pockley AG, Wu R, Lemne C, et al. Circulating heat shock protein 60 is associated with early cardiovascular disease. *Hypertension*. 2000;36(2):303–307.
27. Davies EL, Bacelar MM, Marshall MJ, et al. Heat shock proteins form part of a danger signal cascade in response to lipopolysaccharide and GroEL. *Clin Exp Immunol*. 2006;145(1):183–189.
28. Guzhova I, Kislyakova K, Moskaliova O, et al. In vitro studies show that Hsp70 can be released by glia and that exogenous Hsp70 can enhance neuronal stress tolerance. *Brain Res*. 2001;914(1-2):66–73.
29. Hunter-Lavin C, Davies EL, Bacelar MM, et al. Hsp70 release from peripheral blood mononuclear cells. *Biochem Biophys Res Commun*. 2004;324(2):511–517.
30. Ireland HE, Leoni F, Altaie O, et al. Measuring the secretion of heat shock proteins from cells. *Methods*. 2007;43(3):176–183.
31. Hennekens CW. Increasing global burden of cardiovascular disease in general populations and patients with schizophrenia. *J Clin Psychiatry*. 2007;68(suppl 4):4–7.
32. Khan MM, Evans DR, Gunna V, et al. Reduced erythrocyte membrane essential fatty acids and increased lipid peroxides in schizophrenia at the never-medicated first-episode of psychosis and after years of treatment with antipsychotics. *Schizophr Res*. 2002;58(1):1–10.
33. Neeman G, Blanaru M, Bloch B, et al. Relation of plasma glycine, serine, and homocysteine levels to schizophrenia symptoms and medication type. *Am J Psychiatry*. 2005;162(9):1738–1740.
34. Ramchand CN, Davies JI, Tresman RL, et al. Reduced susceptibility to oxidative damage of erythrocyte membranes from medicated schizophrenic patients. *Prostaglandins Leukot Essent Fatty Acids*. 1996;55(1–2):27–31.
35. Zhang XY, Tan YL, Cao LY, et al. Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia treated with typical and atypical antipsychotics. *Schizophr Res*. 2006;81(2–3):291–300.
36. Bonelli RM, Hofmann P, Aschoff A, et al. The influence of psychotropic drugs on cerebral cell death: female neurovulnerability to antipsychotics. *Int Clin Psychopharmacol*. 2005;20(3):145–149.
37. Abdalla DS, Monteiro HP, Oliveira JA, et al. Activities of superoxide

dismutase and glutathione peroxidase in schizophrenic and manic-depressive patients. *Clin Chem*. 1986;32(5):805–807.
38. Akyol Ö, Herken H, Uz E, et al. The indices of endogenous oxidative and antioxidative processes in plasma from schizophrenic patients: the possible role of oxidant/antioxidant imbalance. *Prog Neuropsychopharmacol Biol Psychiatry*. 2002;26(5):995–1005.
39. Evans DR, Parikh VV, Khan MM, et al. Red blood cell membrane essential fatty acid metabolism in early psychotic patients following antipsychotic drug treatment. *Prostaglandins Leukot Essent Fatty Acids*. 2003;69(6):393–399.
40. Kim JJ, Lee SJ, Toh KY, et al. Identification of antibodies to heat shock proteins 90 kDa and 70 kDa in patients with schizophrenia. *Schizophr Res*. 2001;52(1–2):127–135.
41. Linday LA, Pippenger CE, Howard A, et al. Free radical scavenging enzyme activity and related trace metals in clozapine-induced agranulocytosis: a pilot study. *J Clin Psychopharmacol*. 1995;15(5):353–360.
42. Bindoli A, Rigobello MP, Cavallini L, et al. Decrease of serum malondialdehyde in patients treated with chlorpromazine. *Clin Chim Acta*. 1987; 169(2–3):329–332.
43. Gama CS, Salvador M, Andreazza AC, et al. Elevated serum superoxide dismutase and thiobarbituric acid reactive substances in schizophrenia: a study of patients treated with haloperidol or clozapine. *Prog Neuropsychopharmacol Biol Psychiatry*. 2006;30(3):512–515.
44. Jeding I, Evans PJ, Akanmu D, et al. Characterization of the potential antioxidant and pro-oxidant actions of some neuroleptic drugs. *Biochem Pharmacol*. 1995;49(3):359–365.
45. Kropp S, Kern V, Lange K, et al. Oxidative stress during treatment with first- and second-generation antipsychotics. *J Neuropsychiatry Clin Neurosci*. 2005;17(2):227–231.
46. Pai BN, Janakiramaiah N, Gangadhar BN, et al. Depletion of glutathione and enhanced lipid peroxidation in the CSF of acute psychotics following haloperidol administration. *Biol Psychiatry*. 1994;36(7):489–491.
47. Peet M, Laugharne J, Rangarajan N, et al. Depleted red cell membrane essential fatty acids in drug-treated schizophrenic patients. *J Psychiatr Res*. 1995;29(3):227–232.
48. Sarandol A, Kirli S, Akkaya C, et al. Oxidative-antioxidative systems and their relation with serum S100 B levels in patients with schizophrenia: effects of short term antipsychotic treatment. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007;31(6):1164–1169.
49. Arolt V, Rothermundt M, Wandinger KP, et al. Decreased in vitro production of interferon-gamma and interleukin-2 in whole blood of patients with schizophrenia during treatment. *Mol Psychiatry*. 2000;5(2):150–158.
50. Kamińska T, Szuster-Ciesielska A, Wysocka A, et al. Serum cytokine level and production of reactive oxygen species (ROS) by blood neutrophils from a schizophrenic patient with hypersensitivity to neuroleptics. *Med Sci Monit*. 2003;9(7):CS71–CS75.
51. Yao JK, Reddy R, McElhinny LG, et al. Effects of haloperidol on antioxidant defense system enzymes in schizophrenia. *J Psychiatr Res*. 1998; 32(6):385–391.
52. Zhang X, Zhou D, Cao L, et al. Pharmacokinetic profile of an oral loading dose of divalproex sodium during acute alcohol withdrawal. *J Clin Psychopharmacol*. 2006;26:104–105.
53. Skoblenick KJ, Castellano JM, Rogoza RM, et al. Translocation of AIF in the human and rat striatum following protracted haloperidol, but not clozapine treatment. *Apoptosis*. 2006;11(5):663–672.
54. Zhang XY, Zhou DF, Su JM, et al. The effect of extract of ginkgo biloba added to haloperidol on superoxide dismutase in inpatients with chronic schizophrenia. *J Clin Psychopharmacol*. 2001;21(1):85–88.
55. Kim YK, Suh IB, Kim H, et al. The plasma levels of interleukin-12 in schizophrenia, major depression, and bipolar mania: effects of psychotropic drugs. *Mol Psychiatry*. 2002;7(10):1107–1114.
56. Lieber MR, Lange Y, Weinstein RS, et al. Interaction of chlorpromazine with the human erythrocyte membrane. *J Biol Chem*. 1984;259(14): 9225–9234.
57. Rudolf S, Peters M, Rothermundt M, et al. The influence of typical and atypical neuroleptic drugs in the production of interleukin-2 and interferon-gamma in vitro. *Neuropsychobiology*. 2002;46(4):180–185.
58. Szuster-Ciesielska A, Słotwińska M, Stachura A, et al. Neuroleptics modulate cytokine and reactive oxygen species production in blood leukocytes of healthy volunteers. *Arch Immunol Ther Exp (Warsz)*. 2004;52(1):59–67.
59. Arvindakshan M, Sitasawad S, Debsikdar V, et al. Essential polyunsaturated fatty acid and lipid peroxide levels in never-medicated and medicated schizophrenia patients. *Biol Psychiatry*. 2003;53(1):56–64.
60. Gross A, Joffe G, Joutsiniemi SL, et al. Decreased production of reactive oxygen species by blood monocytes caused by clozapine correlates with EEG slowing in schizophrenic patients. *Neuropsychobiology*. 2003;47(2): 73–77.
61. Joffe G, Nyberg P, Gross A, et al. Clozapine-induced decrease in the production of reactive oxygen metabolites by monocytes in vitro may predict clinical response to clozapine in treatment-resistant schizophrenia. *Hum Psychopharmacol*. 1999;14(3):203–209.
62. Dakhale G, Khanzode S, Khanzode S, et al. Oxidative damage and schizophrenia: the potential benefit by atypical antipsychotics. *Neuropsychobiology*. 2004;49(4):205–209.
63. Fehsel K, Loeffler S, Krieger K, et al. Clozapine induces oxidative stress and proapoptotic gene expression in neutrophils of schizophrenic patients. *J Clin Psychopharmacol*. 2005;25(5):419–426.
64. Joffe G, Nyberg P, Gross A, et al. Is there an association between the effects of clozapine on the production of reactive oxygen metabolites by blood monocytes and clinical outcome in neuroleptic-resistant schizophrenia? *Hum Psychopharmacol*. 1998;13(4):231–237.
65. Zhang XY, Zhou DF, Cao LY, et al. The effect of risperidone treatment on superoxide dismutase in schizophrenia. *J Clin Psychopharmacol*. 2003;23(2):128–131.
66. Atmaca M, Tezcan E, Kuloglu M, et al. The effect of extract of ginkgo biloba addition to olanzapine on therapeutic effect and antioxidant enzyme levels in patients with schizophrenia. *Psychiatry Clin Neurosci*. 2005;59(6):652–656.
67. Maes M, Meltzer HY, Bosmans E. Immune-inflammatory markers in schizophrenia: comparison to normal controls and effects of clozapine. *Acta Psychiatr Scand*. 1994;89(5):346–351.
68. Hinze-Selch D, Becker EW, Stein GM, et al. Effects of clozapine on in vitro immune parameters: a longitudinal study in clozapine-treated schizophrenic patients. *Neuropsychopharmacology*. 1998;19(2):114–122.
69. Hinze-Selch D, Deuschle M, Weber B, et al. Effect of coadministration of clozapine and fluvoxamine versus clozapine monotherapy on blood cell counts, plasma levels of cytokines and body weight. *Psychopharmacology (Berl)*. 2000;149(2):163–169.
70. Pollmächer T, Hinze-Selch D, Mullington J. Effects of clozapine on plasma cytokine and soluble cytokine receptor levels. *J Clin Psychopharmacol*. 1996;16(5):403–409.
71. Mahadik SP, Pillai A, Joshi S, et al. Prevention of oxidative stress-mediated neuropathology and improved clinical outcome by adjunctive use of a combination of antioxidants and omega-3 fatty acids in schizophrenia. *Int Rev Psychiatry*. 2006;18(2):119–131.
72. Schwarz MJ, Riedel M, Gruber R, et al. Antibodies to heat shock proteins in schizophrenic patients: implications for the mechanism of the disease. *Am J Psychiatry*. 1999;156(7):1103–1104.
73. Dietrich-Muszalska A, Olas B. Isoprostenes as indicators of oxidative stress in schizophrenia. *World J Biol Psychiatry*. 2009;10(1):27–33.
74. Ahmed N, Babaei-Jadidi R, Howell SK, et al. Degradation products of proteins damaged by glycation, oxidation and nitration in clinical type 1 diabetes. *Diabetologia*. 2005;48(8):1590–1603.