International Journal of Neuropsychopharmacology (2008), **11**, 1097–1104. Copyright © 2008 CINP
doi:10.1017/S1461145708008882

ARTICLE

CINP

Clozapine causes oxidation of proteins involved in energy metabolism: a possible mechanism for antipsychotic-induced metabolic alterations

Consuelo Walss-Bass[1], Susan T. Weintraub[2], John Hatch[1,3], Jim Mintz[1] and Asish R. Chaudhuri[2]

[1] *Department of Psychiatry,* [2] *Department of Biochemistry,* [3] *Department of Orthodontics, The University of Texas Health Science Center at San Antonio, TX, USA*

Abstract

Although atypical antipsychotics are widely known to induce alterations in lipid and glucose metabolism, the mechanisms by which these alterations occur remain unknown. Several recent studies have shown that atypical antipsychotics induce oxidative stress and oxidative cell injury by increasing levels of lipid and protein oxidation. In this study, a novel proteomic approach was used to identify specific proteins oxidized after clozapine treatment. Differentiated neuroblastoma SKNSH cells were treated with 0, 5 or 20 μM clozapine for 24 h and protein extracts were labelled with 6-iodoacetamide fluorescein (6-IAF). The lack of incorporation of 6-IAF to cysteine residues is an indicator of protein oxidation. Labelled proteins were exposed to 2D electrophoresis, and differential protein labelling was assessed. Increased oxidation after clozapine treatment was observed in 10 protein spots ($p < 0.05$), although only four of them remained significant after correcting for analysis with two drug concentrations. Five proteins, corresponding to nine of the spots, were identified by HPLC–electrospray ionization tandem mass spectrometry (HPLC–ESI–MS/MS) as mitochondrial ribosomal protein S22, mitochondrial malate dehydrogenase, calumenin, pyruvate kinase and 3-oxoacid CoA transferase. The latter four proteins play important roles in energy metabolism. These results suggest that oxidative stress may be a mechanism by which antipsychotics increase the risk for metabolic syndrome and diabetes.

Received 18 December 2007; Reviewed 10 February 2008; Revised 26 March 2008; Accepted 30 March 2008; First published online 9 May 2008

Key words: Glucose metabolism, oxidative stress, proteomics, reactive oxygen species.

Introduction

Antipsychotics are widely used for the treatment of many psychiatric disorders due to their ability to alleviate positive symptomatology (hallucinations and delusions). However, despite years of intensive research, the biological mechanisms underlying their therapeutic properties remain largely unknown. In fact, our understanding of the mode of action of these drugs goes little beyond the targeting of neurotransmitter receptors. The typical antipsychotics are known to act primarily as D_2 dopamine receptor antagonists. The high affinity of these drugs for the dopamine receptor is associated with increased risk of extrapyramidal symptoms such as tardive dyskinesia (Galili et al., 2000). The atypical antipsychotics have relatively lower affinity for the dopamine D_2 receptors and higher affinity for other receptors, including the serotonin, muscarinic and histamine receptors (Miyamoto et al., 2005). Even though atypical antipsychotics are associated with fewer extrapyramidal symptoms, they are known to cause other severe side-effects such as weight gain, hyperglycaemia and metabolic alterations (Haupt, 2006; Newcomer, 2005; Rettenbacher, 2005). Although the biological mechanisms through which these side-effects occur remain unknown, it is known that different atypical antipsychotics differ in their risk to cause these side-effects. Clozapine and olanzapine are associated with greater weight gain compared to quetiapine, risperidone, ziprasidone and aripiprazole, with ziprasidone and aripiprazole causing the least weight gain.

Address for correspondence: C. Walss-Bass, Ph.D., 7703 Floyd Curl Drive, San Antonio, TX 78229, USA.
Tel.: 210-567-5461 *Fax*: 210-567-3759
E-mail: walss@uthscsa.edu

Downloaded from https://academic.oup.com/ijnp/article/11/8/1097/695962 by guest on 30 April 2025

Likewise, clozapine and olanzapine are associated with a greater risk for hyperglycaemia, ketoacidosis and insulin resistance, compared to the other atypicals (Newcomer, 2005; Rettenbacher, 2005). A recent study in rats showed that changes in insulin resistance can occur after a single-dose treatment with either olanzapine or clozapine, but not ziprasidone, indicating that antipsychotics may have a direct effect in glucose metabolism, in the absence of weight gain (Houseknecht et al., 2007).

Oxidative stress and the concomitant production of reactive oxygen species (ROS) have been implicated in the clinical side-effects of antipsychotics. Both haloperidol and clozapine induce oxidative stress in rat brain. Decreased levels of antioxidant enzymes and increased membrane lipid peroxidation (caused by ROS) occur following treatment with these drugs (Polydoro et al., 2004; Reinke et al., 2004). These same alterations occur after long-term treatment with other atypical antipsychotics (risperidone, and ziprasidone) in rats (Pillai et al., 2007).

Oxidative stress has been implicated in the pathogenesis of both type 1 and type 2 diabetes (Kaneto et al., 2007). It is possible that antipsychotic-induced oxidative stress may be an underlying factor in the mechanism by which antipsychotics elicit increased risk for adverse metabolic side- effects.

The thiol (–SH) group in the amino acid cysteine is particularly sensitive to oxidation and can be oxidized to several states including irreversible oxidation states [sulfinic (SO_2H) and sulfonic (SO_3H) acids]. In the present study we used a proteomic approach to perform an unbiased search of proteins that are irreversibly oxidized by treatment with clozapine, as representative of atypical antipsychotics, in order to elucidate the molecular pathways by which this drug causes its metabolic side-effects.

Methods

Cell culture

SKNSH neuroblastoma cells (ATCC, Manassas, VA, USA) were grown as previously described (Pizzi et al., 2002). Briefly, cells were plated at 37 °C in Dulbecco's Modified Eagle's Medium supplemented with 4 mM glutamine. Cells were allowed to differentiate to a neuronal phenotype by incubation with 10 μm retinoic acid for 2 wk.

Measurement of ROS

Differentiated SKNSH cells were treated with clozapine (Sigma-Aldrich, St Louis, MO, USA) (1, 5, 10 or 20 μM) or vehicle (ethanol only) for 24 h, respectively. Experiments were performed in triplicate with all drug concentrations. Cell pellets were collected and incubated with 10 μM dichlorofluorescin diacetate (DCFH-DA, Invitrogen, Carlsbad, CA, USA) in PBS for 20 min at 37 °C. This non-fluorescent compound is converted to fluorescent dichlorofluorescein (DCF) upon oxidation by peroxides. Therefore, this probe is widely used to detect levels of ROS in cells (Sagara, 1998). Fluorescence intensity levels were measured using an excitation wavelength of 475 nm and an emission wavelength of 525 nm.

Cell viability and apoptosis assays

The number of live and dead cells was determined by incubating the treated cells (clozapine-treated and controls) with Trypan Blue for 5 min and placing a fraction of the cells in a Neubauer haemocytometer and counting the number of cells that either incorporated or excluded the dye (dead or alive respectively).

Apoptosis was assayed by the terminal deoxynucleotide-transferase-mediated d-UTP-biotin nick end-labelling (TUNEL) assay using an in-situ cell death detection kit obtained from R&D Systems (Minneapolis, MN, USA) and performed according to manufacturer's instructions. All assays were performed in triplicate at each drug concentration (5 μM and 20 μM clozapine) and compared to vehicle (ethanol alone)

Labelling of protein thiols by 6-iodoacetamidofluorescein (6-IAF)

Differentiated cells were treated with clozapine (5 μM and 20 μM) or vehicle (ethanol only) for 24 h, respectively. A 10 mM drug stock was freshly prepared by dissolving in ethanol. The amount of ethanol added was equal in all samples ($<0.5\%$ in all samples). Experiments were performed in triplicate with all drug concentrations. Protein extracts were obtained by sonication of cells in buffer containing 50 mM potassium phosphate (pH 7.9), 0.5 mM $MgCl_2$, 1 mM EDTA, Halt protease inhibitor cocktail (Pierce, Rockford, IL, USA) and were then centrifuged at 6000 g at 4 °C for 1 h (Pierce et al., 2007). The supernatant was treated with 6 M urea at 37 °C for 1 h followed by treatment with 2 mM DTT and sodium arsenite (20 mM) to reduce the disulfide (S–S) and sulfenic acid (SOH), respectively (Saurin et al., 2004). In this context, it should be noted that sodium arsenite can not reduce sulfinic and sulfonic acids; as such these modifications are considered to be irreversible.

Downloaded from https://academic.oup.com/ijnp/article/11/8/1097/695962 by guest on 30 April 2025

The extract was then treated with 5 mM 6-IAF (Invitrogen, Carslbad, CA, USA) and incubated at 37 °C for 1 h in the dark for complete reaction between thiols and 6-IAF. The proteins were then precipitated with an equal volume of 20% TCA, centrifuged at 12 000 *g* for 20 min and washed at least three times with ethanol/ethylacetate (1:1, v/v) to remove urea, DTT, sodium arsenite and free 6-IAF. 6-IAF-labelled protein pellets were then dissolved in 8 M urea, 2% Chaps, 0.5% IPG buffer (GE Healthcare, Piscataway, NJ, USA) and DeStreak reagent (15 mg/ml, GE Healthcare); protein concentrations were assessed through the use of a BCA assay (Pierce).

2D gel electrophoresis

The labelled protein extracts were subjected to 2D gel electrophoresis as follows: 100 μg of protein was subjected to isoelectric focusing overnight on 13 cm strips (pH 3–10) using the IPGphor system (GE Healthcare). After the first dimension, strips were equilibrated for 15 min in buffer containing first DTT (100 mg/ml) and then iodoacetamide (250 mg/ml). The second dimension was then run using precast Criterion 12% SDS–PAGE gels (Bio-Rad, Hercules, CA, USA), after which 6-IAF fluorescence was assessed using a Typhoon 9400 variable mode imager (GE Healthcare) with excitation at 490 nm. Gels were then stained with SYPRO Ruby (Invitrogen) overnight, after which they were washed twice with water and then soaked in water for 15 min. Gel images were then acquired using the Typhoon 9400 with excitation at 532 nm.

Quantitative assessment of gel images

Spot intensities on 6-IAF and SYPRO Ruby gel images were quantified separately using Imagequant version 5.0 software (GE Healthcare). To obtain the 6-IAF fluorescence relative to total protein fluorescence, the pixel intensity/area of 6-IAF fluorescence was divided by the pixel intensity/area of SYPRO Ruby fluorescence for each spot. Because 6-IAF does not bind to irreversibly oxidized cysteine residues, a decrease in fluorescence intensity in protein spots of treated compared to untreated samples is an indication of greater oxidation (i.e. less labelling of 6-IAF). To identify protein spots that showed statistically significant ($p < 0.05$) mean differences in fluorescence intensity as a function of clozapine dose, we compared the 5 μM and 20 μM conditions to the control (cells treated with vehicle only) using *t* tests for independent samples with two-sided significance testing and we performed Dunnett's tests for group vs. control at adjusted $p = 0.05$ to correct for testing two drug concentrations.

Protein identification by mass spectrometry

Spots exhibiting significant differences in 6-IAF fluorescence were excised from the gels and digested in situ with trypsin. The resulting digests were subjected to capillary HPLC–electrospray ionization tandem mass spectrometry (HPLC–ESI–MS/MS) using a Thermo Fisher LTQ ion trap mass spectrometer interfaced to an Eksigent NanoLC micro HPLC system (Eksigent, Dublin, CA, USA) via a New Objective (Woburn, MA, USA) PicoFrit nanospray interface. Online HPLC separation of the proteolytic peptides was accomplished as follows: column, PicoFrit (New Objective; 75 μm i.d.) packed to 10 cm with C18 adsorbent (Vydac, 218MSB5; 5 μm, 300 Å); mobile phase A, 0.5% acetic acid/0.005% TFA; mobile phase B, 90% acetonitrile/0.5% acetic acid/0.005% trifluoroacetic acid; linear gradient of 2–42% B in 30 min; flow rate, 0.4 μl/min. As part of the data-dependent acquisition protocol, the seven most intense ions in each survey scan were sequentially fragmented in the ion trap by collision-induced dissociation using an isolation width of 3.5 and a relative collision energy of 35%. Uninterpreted tandem mass spectra was searched against published databases using Mascot (Matrix Science, Boston, MA, USA; 10 processor in-house license). Cross-correlation of the Mascot results with X! Tandem and determination of probabilities of protein and peptide identifications were made by Scaffold (Proteome Software, Portland, OR, USA). Assignment of the tandem MS fragments were verified by comparison with the predicted ions generated in silico by GPMAW (Lighthouse Data, Denmark).

Results

Clozapine-induced production of ROS in neuroblastoma cells

To investigate if treatment of differentiated neuroblastoma cells with clozapine led to an increase in ROS levels, differentiated SKNSH cells were treated with clozapine (1, 10 or 20 μM) or vehicle (ethanol only) for 24 h, respectively. Experiments were performed in triplicate for all drug concentrations. Oxidation of DCFH-DA by peroxides was assayed by measuring the fluorescence intensity of the fluorescent oxidized compound (DCF) as a function of drug concentration. The results in Figure 1 show that clozapine exhibited a dose-dependent pattern of ROS generation: ROS levels were seen to increase from baseline after

Downloaded from https://academic.oup.com/ijnp/article/11/8/1097/695962 by guest on 30 April 2025

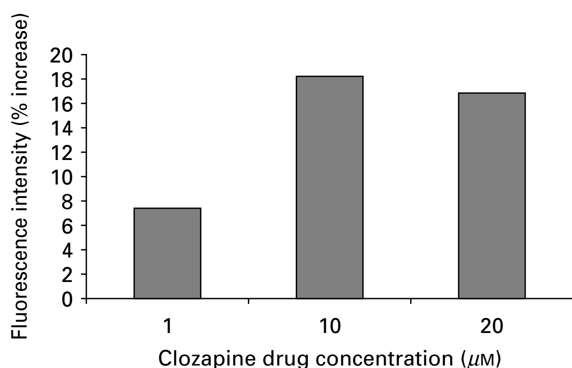

Figure 1. Generation of ROS in SKNSH cells by clozapine treatment. Cells were treated with 0–20 μM drug for 24 h and the levels of ROS generation were determined by using DCFH-DA, as described. Experiments were performed in triplicate for each drug concentration. Each bar represents the percent increase of the mean of the fluorescence intensities when compared to untreated cells.

treatment with 1 μM and reached maximum levels at 10 μM (18% increase). However, the concentrations of clozapine used did not cause significant differences in cell viability or apoptosis in SKNSH cells after 24 h of treatment. The percentage of dead cells was 10.7, 11.4 and 18.5% for 0, 5 and 20 μM clozapine respectively, as measured by the Trypan Blue assay. The TUNEL indices were 0.109 ± 0.012, 0.098 ± 0.014 and 0.092 ± 0.001 in 0, 5 and 20 μM clozapine respectively. These results indicate that SKNSH cells are sensitive to clozapine-induced oxidative stress, without an appreciable effect in cell viability.

Differences in protein oxidation

SKNSH neuroblastoma cells were differentiated to cells with a neuronal phenotype by incubation with 10 μm retinoic acid. Differentiated cells were treated with clozapine (vehicle only, 5 μM and 20 μM) for 24 h, respectively. Experiments were performed in triplicate for all drug concentrations. These concentrations were chosen based on the results obtained above, that they would induce generation of ROS. Protein extracts were obtained and proteins were incubated with the fluorescent probe 6-IAF and subjected to 2D gel electrophoresis as described in the Methods section, after which an image of the gels was obtained. Gels were then treated with SYPRO Ruby to assess total protein, and a second gel image was obtained. A total of 153 spots labelled with SYPRO Ruby were matched across all nine gels [three control (vehicle only) gels, three low-drug concentration and three high-drug concentration]. Figure 2 shows gel images for 6-IAF and SYPRO Ruby fluorescence in a representative 2D gel electrophoresis separation of proteins isolated from SKNSH cells.

Differences in 6-IAF incorporation in individual proteins were then assessed by calculating the ratio of 6-IAF/SYPRO Ruby fluorescence in each of the spots seen in the gels before and after treatment with drug. Statistical analyses using *t* tests for independent samples revealed significant changes ($p<0.05$) in fluorescence intensity in 10 protein spots (73, 79, 91, 110, 118, 119, 120, 124, 125, 126), at either the 5 μM or 20 μM dose. After correcting for testing of two drug doses, only four spots (79, 119, 120 and 124) remained statistically significant at $p<0.05$. All of these spots indicated greater irreversible protein oxidation (reduced 6-IAF fluorescence) at both doses compared to controls. However, only spot 79 exhibited a significant difference after treatment with the 5 μM dose. The other three spots exhibited significant differences only after treatment with the 20 μM dose. Figure 3 shows an expanded view of a selected protein spot that exhibited altered 6-IAF labelling after treatment with clozapine.

Identification of oxidized proteins

We have identified 9 of the 10 spots that exhibited altered oxidation when treated with clozapine (as assessed by 6-IAF labelling) by using HPLC–ESI–MS/MS. These nine spots corresponded to five different proteins, as some of the spots (118, 119, 120 and 124, 125, 126) were identified as the same protein which may represent different post-translational modifications. The proteins with the highest number of identified unique and total spectra are listed in Table 1. Additional minor proteins identified are listed in Table 2.

Discussion

We used a proteomic approach to identify proteins in differentiated SKNSH cells that are oxidized after treatment with the antipsychotic clozapine. The overall concept is to identify *irreversible* protein oxidation by using a modification of a cysteine-labelling method initially described by Baty et al. (2002). Cysteine residues are remarkably sensitive markers of cellular oxidation status. In the approach used for our studies, the level of formation of irreversibly oxidized thiols (conversion of thiols in proteins to either sulfinic or sulfonic acids) is measured by quantifying the incorporation of 6-IAF to the cysteine residues of proteins. The reaction chemistry of 6-IAF is specific for free thiol

Downloaded from https://academic.oup.com/ijnp/article/11/8/1097/695962 by guest on 30 April 2025

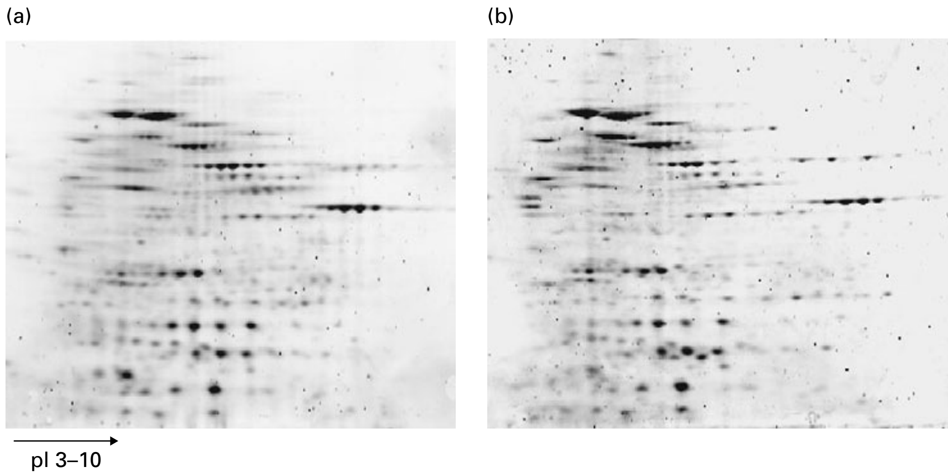

Figure 2. 2D gel electrophoresis of proteins from SKNSH cells. (a) 6-IAF-labelled proteins, (b) the same gel imaged after staining with SYPRO Ruby (total protein); 100 μg of protein was used for each gel.

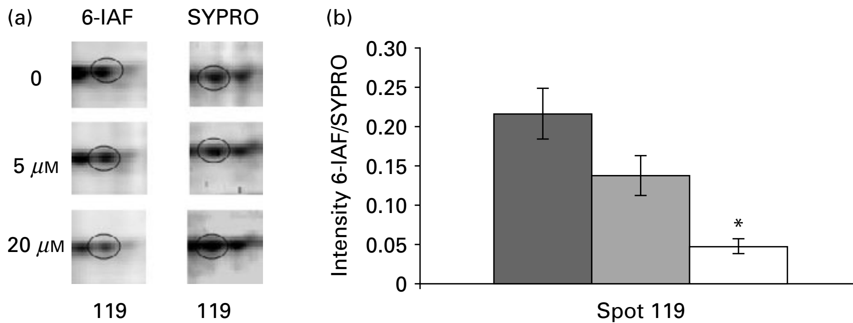

Figure 3. Enlargement of a region containing a protein spot (119) that showed changes in 6-IAF fluorescence intensity after treatment with clozapine. (a) Gel images of cells treated with 0 μM (■), 5 μM (■) and 20 μM (□) clozapine. (b) Plot of mean ± S.D. fluorescence intensity changes as a function of drug concentration. Fluorescence intensity is reported as the ratio of spot intensity of 6-IAF/SYPRO Ruby. Protein spot 119 corresponds to 3-oxoacid CoA transferase (see Table 1) (* $p<0.05$).

(–SH), and therefore, 6-IAF can not react with any oxidized form of cysteines. The loss of incorporation of 6-IAF to the cysteine residues of proteins, after all disulfide bonds and sulfenic acids have been reduced, is the indication of irreversible oxidation. Labelling of cysteine residues has been used in recent studies to detect protein oxidation using a proteomics approach (Pierce et al., 2007; Zhang et al., 2007).

Alterations in protein oxidation could be caused by generation of free radicals and ROS by drug-induced oxidative stress. Sagara (1998) initially showed, using cultured neuronal cells, that haloperidol induces accumulation of ROS in neurons, leading to increased levels of intracellular Ca^{2+} and cell death. More recent studies show that both typical and atypical antipsychotics induce membrane lipid peroxidation and total protein oxidation (Pillai et al., 2007; Polydoro et al., 2004; Reinke et al., 2004). To our knowledge, this is the first study to directly identify proteins that are oxidized by treatment with antipsychotic drugs.

For the present study we used cultured differentiated neuroblastoma cells as a model to identify proteins that are affected by antipsychotic-induced oxidative stress after treatment with clozapine. Differentiated neuroblastomas express many of the neurotransmitter receptors that are targeted by antipsychotics (Pizzi et al., 2002). Given that this was an exploratory study, we chose to use two different drug concentrations (low and high), in order to determine dose effects on protein oxidation. Increased protein oxidation (decreased 6-IAF fluorescence) was observed for 10 protein spots ($p<0.05$), although for eight of the spots significant differences were only achieved after treatment with the 20 μM dose.

Downloaded from https://academic.oup.com/ijnp/article/11/8/1097/695962 by guest on 30 April 2025

Table 1. Proteins exhibiting increased oxidation in differentiated SKNSH cells treated with clozapine

Spot no.	Protein	Accession no.[b]	Fold change		Identification by MS[a]		
					Spectra		
			5 μM	20 μM	Total	Unique	Sequence coverage (%)
73[c]	Mitochondrial malate dehydrogenase	AAH01917	−2.22	−1.98	91	32	43
79	Calumenin	AAK72908	−3.39	−1.66	55	23	26
91[c]	Mitochondrial ribosomal protein S22	AAH09296	−1.57	−2.12	10	4	12
119	3-oxoacid CoA transferase	NP000427	−1.41	−2.40	16	11	18
124	Pyruvate kinase muscle	AAH07640	−1.26	−2.19	30	5	14

[a] Protein spots were digested in situ with trypsin and analysed by HPLC–ESI–MS/MS as described in the Methods section. Identifications were obtained by searching the ?NCBInr database by means of Mascot. Cross-correlation of the Mascot results with X! Tandem and determination of protein and peptide identification probabilities was obtained with Scaffold. All peptides listed had a ⩾95% probability of identification, and all proteins a 100% probability, as determined by Scaffold.
[b] NCBInr accession number.
[c] The observed increase in oxidation was only significant before correcting for analysis with two drug concentrations.

Table 2. Additional proteins identified by HPLC–ESI–MS/MS

Spot no.	Protein	Identification by MS[a]		
		Spectra		
		Total	Unique	Sequence coverage (%)
73	Human muscle L-lactate dehydrogenase	9	3	9
79	Actin	3	3	7
119	Cytosol aminopeptidase	12	9	12
	UDP-glucose dehydrogenase	10	5	8
	Histidyl-tRNA synthetase	9	6	7
	Fascin-1	8	5	8
	MLL septin-like fusion	8	5	8
	Tryptophanyl-tRNA synthetase	6	6	11
	Peptidase D	3	3	5
124	Acute morphin dependence-related protein 2	8	5	7
	Tyrosyl-tRNA synthetase	8	4	7
	Pyrroline-5-carboxylate dehydrogenase	6	4	2

[a] Protein spots were digested in situ with trypsin and analysed by HPLC–ESI–MS/MS as described in the Methods section. Identifications were obtained by searching the ?NCBInr database by means of Mascot. Cross-correlation of the Mascot results with X! Tandem and determination of protein and peptide identification probabilities was obtained with Scaffold. All peptides listed had a 95% probability of identification, and all proteins a 100% probability, as determined by Scaffold.

Using HPLC–ESI–MS/MS we identified proteins for 9 of the 10 spots exhibiting significant differences in 6-IAF fluorescence (Table 1). Four of the identified proteins are involved in energy metabolism. Mitochondrial malate dehydrogenase is a key enzyme in the malate shuttle system that catalyses the conversion

Downloaded from https://academic.oup.com/ijnp/article/11/8/1097/695962 by guest on 30 April 2025

of malate to oxaloacetate. Antipsychotic-induced changes in expression of malate dehydrogenase have been reported by both cDNA microarray and proteomic studies (La et al., 2006; Middleton et al., 2002). Calumenin is a calcium-binding protein that regulates activation of several enzymes, including phosphorylase kinase and glycogen synthase, the enzymes involved in regulation of glycogen breakdown and synthesis. Given that calumenin is present at very low concentrations in the brain, the irreversible oxidation of this protein may be very significant in terms of energy metabolism and other cellular processes. Pyruvate kinase catalyses the final reaction of the glycolytic pathway, by converting phosphoenolpyruvate to pyruvate. Olanzapine-induced changes in expression of pyruvate kinase have been reported by cDNA microarray studies (Fatemi et al., 2006). Polymorphisms within the gene coding for pyruvate kinase have been associated with diabetes (Wang et al., 2002a). 3-oxoacid CoA transferase is involved in ketone body metabolism. Mutations of this enzyme are associated with severe ketoacidosis (Fukao et al., 2000; Kassovska-Bratinova et al., 1996). Cases of diabetic ketoacidosis developed during treatment with clozapine have been repeatedly reported (Lund et al., 2001; Sernyak et al., 2003; Wang et al., 2002b). It is possible that clozapine-induced oxidation of the proteins identified in this study could lead to alterations of their function, which in turn could be related to the increased risk of weight gain, hyperglycaemia and ketoacidosis caused by this drug.

Due to the multiple protein spots analysed in the present study and the lack of sophisticated statistical analyses that control for multiple comparisons, it is possible that some of the proteins identified in this study are false positives. However, for this initial screen, we preferred to err on the side of Type I errors (false positives), rather than risk Type II errors (false negatives) which might be more costly if a protein of importance is overlooked. Further studies, preferably using in-vivo models, need to be performed to corroborate the present results.

It is also important to note that, as this was an exploratory study, control drugs were not included in the design. Further studies with other antipsychotics, both typical and atypical, including those which are not known to cause increased risk for weight gain or metabolic syndrome, should be performed in order to determine if the current observed effects are unique to clozapine or to atypical antipsychotics and could therefore indeed be causative of the unique clinical side-effects seen with these drugs.

In summary, we have used a novel proteomic approach to identify proteins that are irreversibly oxidized by clozapine treatment. Our findings of oxidation of enzymes involved in energy metabolism suggest that protein oxidation may be the mechanism by which atypical antipsychotics induce increased risk of metabolic syndrome. Further studies using other atypical antipsychotics are currently underway. Identification of proteins that are structurally altered due to treatment with antipsychotics may lead to design of specific treatments to counteract the serious side-effects caused by administration of these drugs.

Acknowledgements

Mass spectrometry analysis was performed by the UTHSCSA Mass Spectrometry Core Facility. We thank Andrea Nicks and Kevin Hakala for their help with 2D gel electrophoresis and mass spectrometry analysis, respectively. This study was supported by a grant from the Stanley Medical Research Institute to C.W-B.

Statement of Interest

None.

References

Baty JW, Hampton MB, Winterbourn CC (2002). Detection of oxidant sensitive thiol proteins by fluorescence labeling and two-dimensional electrophoresis. *Proteomics 2*, 1261–1266.

Fatemi SH, Reutiman TJ, Folsom TD, Bell C, Nos L, Fried P, Pearce DA, Singh S, Siderovski DP, Willard FS, Fukuda M (2006). Chronic olanzapine treatment causes differential expression of genes in frontal cortex of rats as revealed by DNA microarray technique. *Neuropsychopharmacology 31*, 1888–1899.

Fukao T, Mitchell GA, Song XQ, Nakamura H, Kassovska-Bratinova S, Orii KE, Wraith JE, Besley G, Wanders RJ, Niezen-Koning KE, Berry GT, Palmieri M, Kondo N (2000). Succinyl-CoA: 3-ketoacid CoA transferase (SCOT): cloning of the human SCOT gene, tertiary structural modeling of the human SCOT monomer, and characterization of three pathogenic mutations. *Genomics 68*, 144–151.

Galili R, Mosberg Gil-Ad I, Weizman A, Melamed E, Offen D (2000). Haloperidol-induced neurotoxicity – possible implications for tardive dyskinesia. *Journal of Neural Transmission 107*, 479–490.

Haupt DW (2006). Differential metabolic effects of antipsychotic treatments. *European Journal of Neuropsychopharmacology 16* (Suppl. 3), S149–155.

Houseknecht KL, Robertson AS, Zavadoski W, Gibbs EM, Johnson DE, Rollema H (2007). Acute effects of atypical

Downloaded from https://academic.oup.com/ijnp/article/11/8/1097/695962 by guest on 30 April 2025

antipsychotics on whole-body insulin resistance in rats: implications for adverse metabolic effects. *Neuropsychopharmacology 32*, 289–297.

Kaneto H, Katakami N, Kawamori D, Miyatsuka T, Sakamoto K, Matsuoka TA, Matsuhisa M, Yamasaki Y (2007). Involvement of oxidative stress in the pathogenesis of diabetes. *Antioxidants & Redox Signaling 9*, 355–366.

Kassovska-Bratinova S, Fukao T, Song XQ, Duncan AM, Chen HS, Robert MF, Perez-Cerda C, Ugarte M, Chartrand C, Vobecky S, Kondo N, Mitchell GA (1996). Succinyl CoA: 3-oxoacid CoA transferase (SCOT): human cDNA cloning, human chromosomal mapping to 5p13, and mutation detection in a SCOT-deficient patient. *American Journal of Human Genetics 59*, 519–528.

La Y, Wan C, Zhu H, Yang Y, Chen Y, Pan Y, Ji B, Feng G, He L (2006). Hippocampus protein profiling reveals aberration of malate dehydrogenase in chlorpromazine/clozapine treated rats. *Neuroscience Letters 408*, 29–34.

Lund BC, Perry PJ, Brooks JM, Arndt S (2001). Clozapine use in patients with schizophrenia and the risk of diabetes, hyperlipidemia, and hypertension: a claims-based approach. *Archives of General Psychiatry 58*, 1172–1176.

Middleton FA, Mirnics K, Pierri JN, Lewis DA, Levitt P (2002). Gene expression profiling reveals alterations of specific metabolic pathways in schizophrenia. *Journal of Neuroscience 22*, 2718–2729.

Miyamoto S, Duncan GE, Marx CE, Lieberman JA (2005). Treatments for schizophrenia: a critical review of pharmacology and mechanisms of action of antipsychotic drugs. *Molecular Psychiatry 10*, 79–104.

Newcomer JW (2005). Second-generation (atypical) antipsychotics and metabolic effects: a comprehensive literature review. *CNS Drugs 19* (Suppl. 1), 1–93.

Pierce AP, de Waal E, McManus LM, Shireman PK, Chaudhuri AR (2007). Oxidation and structural perturbation of redox-sensitive enzymes in injured skeletal muscle. *Free Radical Biological Medicine 43*, 1584–1593.

Pillai A, Parikh V, Terry Jr. AV, Mahadik SP (2007). Long-term antipsychotic treatments and crossover studies in rats: differential effects of typical and atypical agents on the expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. *Journal of Psychiatry Research 41*, 372–386.

Pizzi M, Boroni F, Bianchetti A, Moraitis C, Sarnico I, Benarese M, Goffi F, Valerio A, Spano P (2002). Expression of functional NR1/NR2B-type NMDA receptors in neuronally differentiated SK-N-SH human cell line. *European Journal of Neuroscience 16*, 2342–2350.

Polydoro M, Schroder N, Lima MN, Caldana F, Laranja DC, Bromberg E, Roesler R, Quevedo J, Moreira JC, Dal-Pizzol F (2004). Haloperidol- and clozapine-induced oxidative stress in the rat brain. *Pharmacological Biochemical Behavior 78*, 751–756.

Reinke A, Martins MR, Lima MS, Moreira JC, Dal-Pizzol F, Quevedo J (2004). Haloperidol and clozapine, but not olanzapine, induces oxidative stress in rat brain. *Neuroscience Letters 372*, 157–160.

Rettenbacher MA (2005). Disturbances of glucose and lipid metabolism during treatment with new generation antipsychotics. *Current Opinion in Psychiatry 18*, 175–179.

Sagara Y (1998). Induction of reactive oxygen species in neurons by haloperidol. *Journal of Neurochemistry 71*, 1002–1012.

Saurin AT, Neubert H, Brennan JP, Eaton P (2004). Widespread sulfenic acid formation in tissues in response to hydrogen peroxide. *Proceedings of the National Academy of Sciences USA 101*, 17982–17987.

Sernyak MJ, Gulanski B, Leslie DL, Rosenheck R (2003). Undiagnosed hyperglycemia in clozapine-treated patients with schizophrenia. *Journal of Clinical Psychiatry 64*, 605–608.

Wang H, Chu W, Das SK, Ren Q, Hasstedt SJ, Elbein SC (2002a). Liver pyruvate kinase polymorphisms are associated with type 2 diabetes in northern European Caucasians. *Diabetes 51*, 2861–2865.

Wang PS, Glynn RJ, Ganz DA, Schneeweiss S, Levin R, Avorn J (2002b). Clozapine use and risk of diabetes mellitus. *Journal of Clinical Psychopharmacology 22*, 236–243.

Zhang Y, Padalecki SS, Chaudhuri AR, De Waal E, Goins BA, Grubbs B, Ikeno Y, Richardson A, Mundy GR, Herman B (2007). Caspase-2 deficiency enhances aging-related traits in mice. *Mechanisms of Ageing 128*, 213–221.

Downloaded from https://academic.oup.com/ijnp/article/11/8/1097/695962 by guest on 30 April 2025