The influence of psychotropic drugs on cerebral cell death: female neurovulnerability to antipsychotics

Raphael M. Bonelli[a], Peter Hofmann[a], Andreas Aschoff[b], Gerald Niederwieser[c], Clemens Heuberger[d], Gustaf Jirikowski[b] and Hans-Peter Kapfhammer[a]

Tissue transglutaminase (tTG) is a marker for apoptosis, and its protein level is known to be increased in post-mortem Alzheimer's and Huntington's disease brains. tTG is increased in the cerebrospinal fluid of patients with Alzheimer's disease. However, the influence of psychotropic medication on acute cell death has not been studied so far *in vivo*, although some experiments performed *in vitro* suggest that antipsychotic drugs are neurotoxic. The protein level of tTG was examined in the cerebrospinal fluid obtained from 29 patients under neuroleptic medication in the last 24 h before lumbar puncture (eight patients diagnosed with Alzheimer's disease and 21 patients with other neurological diseases), and compared with those from 55 patients without antipsychotic medication (25 Alzheimer's patients and 30 others). In addition, the influence of several other psychotropic drugs on apoptosis was analysed. A significant influence ($P<0.01$) of antipsychotic drugs for both the Alzheimer's and the non-Alzheimer's group was found with respect to tTG protein levels in cerebrospinal fluid. By contrast to the male subgroups, the female groups showed a strong influence of neuroleptics on cerebral cell death. Surprisingly, atypical antipsychotics did not differ from typical neuroleptics in neurotoxicity. By contrast, no influence of antidepressants, cholinesterase-inhibitors, nootropics, tranquilizers and tramadol on cerebral cell death was found. The results suggest that typical and atypical antipsychotic drugs may induce cerebral cell death, especially in female patients. Subjects with Alzheimer's disease might be even more vulnerable to any antipsychotic. Therefore, subsequent research should aim to identify atypical neuroleptics without neurotoxicity. A limit on the use of first- and second-generation antipsychotics in elderly patients is proposed. Finally, the possible connection between the observed increased cerebral cell death and tardive dyskinesia, the most threatening side-effect in antipsychotic therapy, is discussed. *Int Clin Psychopharmacol* 20:145–149 © 2005 Lippincott Williams & Wilkins.

International Clinical Psychopharmacology 2005, 20:145–149

Keywords: Alzheimer's disease, antipsychotic drugs, apoptosis, neurotoxicity, tardive dyskinesia, tissue transglutaminase

[a]University Clinic of Psychiatry, Graz Medical University, Graz, Austria, [b]Institut für Anatomie II, Friedrich Schiller Universität, Jena, Germany, [c]Department of Neurology, Hospital BHB Eggenberg, Graz, Austria and [d]Department of Mathematics, Graz University of Technology, Graz, Austria.

Correspondence and requests for reprints to Raphael M. Bonelli University Clinic of Psychiatry, Graz Medical University, Auenbruggerplatz 31, 8036 Graz, Austria. Tel: +43 316385 86221; fax: +43 316385 3556; e-mail: raphael.bonelli@klinikum-graz.at

Received 6 December 2004 Accepted 28 January 2005

Introduction

Neuroleptics are prescribed worldwide in various indications, mostly in neuropsychiatric disturbances, such as schizophrenia, psychotic depression, behaviour disorders, agitation, delirium and hyperkinetic movement disorders (Tourette syndrome, Huntington's disease). Moreover, these substances are also under use in several other conditions, such as pain, nausea and vomiting, and hiccups disorders (Zitman *et al.*, 1994), although not evidence-based in all indications (Zitman *et al.*, 1991; Patt *et al.*, 1994). It is well known that these substances (typical and atypical) often produce side-effects, with the most prominent of them being tardive dyskinesia. The production of these extrapyramidal unwanted effects is definitely a major limitation to the use of antipsychotics. The pathophysiology of tardive dyskinesia, a disabling and commonly irreversible movement disorder, is still obscure. Current studies performed *in vitro* have focused on the role of neuroleptic-induced neuronal toxicity in its development because the traditional concept of supersensitivity of striatal dopamine receptors is no longer satisfying (Casey, 2004). Haloperidol, the most prominent antipsychotic drug, has been reported to cause cell death *in vitro* (Behl *et al.*, 1995; Noh *et al.*, 2000), probably as a result of oxidative stress, resulting from alternations of mitochondrial function (Cadet *et al.*, 1994; Galili *et al.*, 2000). Chronic treatment with typical neuroleptics most likely lead to brain damage in schizophrenic patients (Sunderland and Cohen, 1987). Neuroimaging studies of tardive dyskinesia brains showed ventricular dilatation and atrophy in several brain areas (Mion *et al.*, 1991; Dalgalarrondo and Gattaz, 1994). Recently, Ukai *et al.* (2004) found that treatment with haloperidol produced apoptosis of cortical neurones accompanied by shrinkage

0268-1315

of the cell body, and prominent DNA ladders, hallmarks of apoptosis.

Tissue transglutaminase (tTG) is known to be an indicator of apoptosis *in vitro* (Volokhina *et al.*, 2003). It is activated during the apoptotic cell death cascade and plays a key role in the formation of apoptotic bodies (Volokhina *et al.*, 2003). tTG is upregulated in apoptotic cells after activation of the caspase cascade *in vitro* (Johnson *et al.*, 1997). On the other hand, inhibition of tTG prevents apoptosis and increases cell survival (Oliverio *et al.*, 1999). An increase in tTG protein level is found in post-mortem Alzheimer's disease (AD) (Lesort *et al.*, 2000) brains, as well as in Huntington's disease (Bailey *et al.*, 2004). In a previous study, our group demonstrated an increased concentration of tTG in the cerebrospinal fluid of AD patients compared to controls, whereas vascular dementia did not differ from controls (Bonelli *et al.*, 2002). We therefore were able to show that tTG may serve as a biochemical marker of apoptosis not only *in vitro*, but also *in vivo*. In the present study, we analysed the influence of psychotropic medication, especially antipsychotic drugs, on cerebrospinal fluid apoptosis.

Methods

The detailed process of selecting and processing cerebrospinal fluid is described elsewhere (Bonelli *et al.*, 2002). We used the cerebrospinal fluid obtained from 29 patients who were under neuroleptic medication in the last 24 h before lumbar puncture, eight of them patients with AD and 21 patients with other neurological diseases. The apoptotic marker was compared with the cerebrospinal fluid of 55 patients without neuroleptic medication, 25 of them diagnosed with AD. All patients underwent lumbar puncture for diagnostic reasons after they had provided their informed consent; no single patient underwent lumbar puncture in the present study. Because controls and vascular dementia do not differ in tTG values (Bonelli *et al.*, 2002), they are united in one group (non-AD) here. The baseline-characteristics of the study subjects are shown in Table 1.

Psychotropic medication (antipsychotics, antidepressants, cholinesterase-inhibitors, nootropics, tranquillizers and tramadol) of all 84 patients was analysed retrospectively from the clinical records. None of the patients received antipsychotic drugs due to a schizophrenic disorder. Demented patients were treated with neuroleptics mostly for behavioural problems, whereas non-demented subjects received these substances for pain relieve in the majority of cases. Due to the hypothesis that older antidepressants might influence brain metabolism differently, the group of antidepressants was finally divided into serotonin reuptake inhibitors (SSRI) and other antidepressants. Final diagnosis at the time of discharge from the hospital and medication at the day before lumbar puncture is shown in Table 2. The Jirikowsi Laboratory at the University of Jena, Germany, carried out the measurement of tTG protein levels by enzyme-linked immunosorbent assay. All laboratory staff were blinded for diagnosis, medication, sex and age of patients; and followed the laboratory method as previously described in detail (Bonelli *et al.*, 2002). A professional statistician performed the statistical analysis of data. Variables are presented as mean ± SD tTG levels. The statistical software package SPSS version 11.5 for windows (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. Because tTG values are non-negative by definition and highly asymmetric, we did not expect them to be normally distributed. A Kolmogorov–Smirnov test rejected the hypothesis of normal distribution. An exponential distribution was also rejected by the Kolmogorov–Smirnov test. Because normal distribution cannot be assumed, we did not confine ourselves to using Student's paired *t*-test (or its variant for samples of heterogeneous variance) alone, although it is known to be rather robust against deviations from normality. We therefore also used Mann–Whitney's non-parametric *U*-test, which does not make any assumptions on normality. However, both tests lead to the same conclusions in all analysis performed. $P < 0.05$ was considered statistically significant. Because cerebrospinal fluid from clinically necessary diagnostic procedures was used retrospectively, no ethic committee approval was necessary in the countries where the study was performed.

Table 1 Baseline characteristics of the study subjects

	AD	Controls
n	33	51
Female (%)	22 (67)	31 (61)
MMSE (mean ± SD)	21.8 ± 4.7[a]	27.3 ± 0.6[a]
Age (mean ± SD)	71.67 ± 8.3[a]	58.63 ± 16.7[a]
APD (%)	10 (30)	21 (41)
SSRI (%)	10 (30)	10 (20)
ADe (%)	17 (52)	18 (35)
CEI (%)	7 (21)[a]	2 (4)[a]
Noo (%)	7 (21)	4 (8)
Ben (%)	4 (12)	9 (18)
Opo (%)	2 (6)	3 (6)
tTG (mean ± SD)	7.57 ± 9.2[a]	2.97 ± 5.1[a]

[a]Statistically significant different. AD, Alzheimer's disease; MMSE, Mini-Mental State Examination; APD, Antipsychotic drugs; SSRI, selective serotonin reuptake inhibitor; ADe, other antidepressive drugs; CEI, choline esterase inhibitor; Noo, nootropic drugs; Ben, bezodiazepines; Opo, opioides (i.e. tramadol); tTG, tissue transglutaminase.

Results

The main results of the study are shown in Table 3. In the AD group, division between neuroleptic medication at the time of lumbar puncture and those without happened to find statistically equally distributed sex-ratios, age, cognitive functioning, SSRI, other antidepressants, cholinesterase-inhibitors and tranquillizers. We found a significant influence ($P < 0.01$) of antipsychotic

Table 2 Diagnosis and medication of subjects

	Total	Female	APD	SSRI	ADe	CEI	Noo	Ben	Tra
AD	33	22	8	10	17	7	7	4	2
VaD	18	11	9	4	9	2	2	2	1
Somatoform disorders	10	6	8	4	6	0	1	0	1
Low back pain	9	4	1	2	2	0	0	0	1
Cephalea	5	3	1	0	0	0	1	0	0
Neuropathy	4	2	1	0	1	0	0	0	0
Meniere's disease	2	2	0	0	0	0	0	0	0
Trigeminal neuralgia	2	2	1	0	0	0	0	0	0
Spasmodic torticollis	1	1	0	0	0	0	0	0	0
Total	84	53	29	20	35	9	11	6	5

AD, Alzheimer's disease; APD, Antipsychotic drugs; SSRI, selective serotonin reuptake inhibitor; ADe, other antidepressive drugs; CEI, choline esterase inhibitor; Noo, nootropic drugs; Ben, bezodiazepines; Tra, tramadol; VaD, vascular dementia.

Table 3 Results from the study

	AD ($n=33$)		Non-AD ($n=51$)	
	nNL	NL	nNL	NL
n	25	8	30	21
Female (%)	16 (64)	6 (75)	23 (77)[a]	6 (29)[a]
MMSE (mean ± SD)	20.4 ± 6.6	22.2 ± 3.9	28.1 ± 3.5	27.3 ± 4.0
Age (mean ± SD)	74.03 ± 6.1	70.91 ± 9.1	58.47 ± 18.4	58.85 ± 14.3
SSRI (%)	8 (32)	2 (25)	4 (13)	6 (29)
ADe (%)	12 (48)	5 (63)	5 (17)[a]	13 (62)[a]
CEI (%)	5 (20)	2 (25)	2 (7)	0 (0)
Noo (%)	4 (16)	3 (38)	0 (0)	2 (10)
Ben (%)	3 (12)	1 (13)	2 (7)	2 (10)
Opo (%)	1 (4)	1 (13)	0 (0)	2 (10)
tTG, men (mean ± SD)	4.67 ± 5.4	10.75 ± 6.4	2.97 ± 1.8	3.13 ± 3.4
tTG, women (mean ± SD)	5.90 ± 7.8[a]	15.37 ± 14.2[a]	0.45 ± 0.8[b]	9.93 ± 9.2[b]
tTG, all (mean ± SD)	5.45 ± 7.0[a]	14.21 ± 12.5[a]	1.04 ± 1.5[a]	5.72 ± 6.9[a]

[a]Statistically significant different.
[b]Highly significant different. MMSE, Mini-Mental State Examination; SSRI, selective serotonin reuptake inhibitor; ADe, other antidepressive drugs; CEI, choline esterase inhibitor; Noo, nootropic drugs; Ben, bezodiazepines; Opo, opioides (i.e. tramadol); NL, group of patients treated with neuroleptics; nNL, group of patients not treated with neuroleptics; AD, group of patients suffering from Alzheimer's disease; non-AD, group of patients not suffering from Alzheimer's disease; tTG, tissue Transglutaminase in the cerebrospinal fluid (in ng/dl).

drugs in the apoptotic marker tTG in this group. Divided by gender, this difference remained statistically significant in females, whereas it failed to show significance in the male subgroup (probably due to small sample size). By contrast, in the non-AD group, neuroleptic-medicated patients included statistically more females and tended to have more (other) antidepressants than those without antipsychotic drugs. Age, cognitive functioning, SSRI, cholinesterase-inhibitors and tranquillizers were equally distributed. In this majoritarian non-demented, younger group with less cholinesterase-inhibitors and a physiologic tTG level (Table 1), we found the same influence of antipsychotic drugs on cerebral apoptosis as in the AD group. Interestingly, the whole effect is driven by the female subgroup, thus changing to highly significance when isolated, whereas males fail to show any apoptotic change by antipsychotic drugs.

Seven different antipsychotic substances were used in our patients. As the next step in our analysis, we divided the subjects to receive four typical neuroleptics (melperone, flupentixol, haloperidol and prothipendyl) and three atypical antipsychotics (risperidone, zotepine, olanzapine). The two groups comprised 15 and 14 patients, respectively, and were equally distributed in sex and diagnosis. Patients receiving typical neuroleptics were significantly younger compared to those taking atypicals (56.7 versus 72.1, respectively). However, tTG is known not to be age-dependent. Astonishingly, despite being equally distributed in the only two important factors (i.e. sex and diagnosis), both groups failed to differ in tTG values (7.81 ng/dl and 8.11 ng/dl, respectively). Of interest is the additional information obtained with respect to the seven single substances: we found five substances to have above average tTG levels compared to the whole cohort of 84 patients (mean value 4.78 ng/dl): melperone (four patients) 14.95 ng/dl; zotepine (four patients) 8.78 ng/dl; olanzapine (eight patients) 8.5 ng/dl; flupentixol (seven patients) 7.86 ng/dl; and haloperidol (one patient) 7.3 ng/dl. By contrast, two substances, risperidone (two patients) 5.25 ng/dl and prothipendyl (three patients) 3.16 ng/dl, did not differ from the average. Nearly all patients free of neuroleptics were actually neuroleptic-naïve. In those five patients with a history of antipsychotic treatment in recent years, but not in the last 24 h before lumbar puncture, tTG levels in

cerebrospinal fluid appeared to be equivalent to their corresponding group without neuroleptics, suggesting a termination of cell death after discontinuation of medication. A multivariant analysis found no influence of SSRI, other antidepressants, cholinesterase-inhibitors, nootropics, tranquilizers and tramadol on the actual tTG level.

Discussion

We found a significant tTG increase in cerebrospinal fluid due to both typical and atypical antipsychotic drugs. These results indicate an increased cerebral apoptotic process, thus suggesting neurotoxicity. Although this is the first report to be obtained *in vivo*, the neurotoxicity of (both typical and atypical) neuroleptics has been found in neuronal cell culture: perphenazine, clozapine and haloperidol decreased cell viability by 87%, 43% and 34%, respectively. On the other hand, risperidone was not toxic in this study (Gil-Ad *et al.*, 2001), thus affirming our results with risperidone and haloperidol. Similarly, haloperidol, but not risperidone, was found to induce caspase-dependent apoptosis by reducing cellular survival signaling (Ukai *et al.*, 2004). Most current findings indicate that haloperidol-induced cell death is apoptotic rather than necrotic (Andreassen and Jorgensen, 2000; Galili *et al.*, 2000; Post *et al.*, 2002). However, it is a rather challenging finding that atypical neuroleptics do not differ from the first-generation antipsychotics in their neurotoxicity, which contradicts the widespread euphoria on these agents. In accordance with our results, Gil-Ad *et al.* (2001) found that an atypical antipsychotic (clozapine) decreased cell viability to a higher degree than the typical neuroleptic haloperidol. Moreover, as the authors pointed out, various atypical substances appear to differ in their potential harmful effects, and we suggest that further studies performed *in vitro* are rewuired for the different types of second-generation antipsychotics.

Our findings in AD patients are challenging and of practical relevance. Seventeen of our demented patients (AD and vascular dementia) received neuroleptics, most of them for behavioural problems (Table 2). These substances still comprise the preferred medication for the management of psychosis or agitation (with or without psychosis) in AD (Cummings, 2004). Atypical neuroleptics are said to produce fewer side-effects, such as parkinsonism and tardive dyskinesia, than conventional neuroleptic drugs. Double-blind, controlled trials support the efficacy of atypical antipsychotics in reducing the rate of psychosis and agitation in patients with AD, as is also known for haloperidol (Cummings, 2004). However, our study failed to show a difference in neurotoxicity between atypical and typical neuroleptics, and we should be extremely careful when using neuroleptic as first-line drugs in AD patients. Beyond doubt, neuroleptics are still necessary in dealing with some agitated demented patients, but therapy with mood stabilizers or antidepressants alone or in combination with antipsychotic agents might be an alternative to reduce neurotoxic burden in this vulnerable patient group.

Our findings in non-AD patients (average age 59 years) are even more challenging. Twelve of our patients received antipsychotics for pain relieve (eight with somatoform disorder, and one patient each with low back pain, cephalea, neuropathy and trigeminal neuropathy, respectively). This off-label treatment is not evidence-based, but nevertheless common in everyday clinical practice (Zitman *et al.*, 1994). We consider it to be an appropriate time to question such subscription practice. Because the level cerebral apoptosis of non-demented patients on antipsychotics appears to be indistinguishable to AD patients without this medication, the question might arise as to whether neuroleptics actually induce some degenerative process. Moreover, the incidence of tardive dyskinesia is much higher in this age group who are receiving antipsychotics than it is in the younger age group. A Canadian study of geriatric psychiatric first-admission patients who had never taken neuroleptic drugs before reported that 35.4% of the patients developed tardive dyskinesia (Yassa *et al.*, 1992), thus confirming the higher vulnerability of non-demented elderly patients treated with neuroleptics.

Moreover, we found a female vulnerability to antipsychotics, both in the AD and especially the non-AD group. Concordantly, prevalence of tardive dyskinesia is significantly higher in women (26.6%) than in men (21.6%) after neuroleptic treatment, as previously reported in a metaanalysis of 39187 patients (Yassa *et al.*, 1992). Interestingly, male AD patients show lower tTG values than females, although this difference did not reach statistical significance, whereas there was no gender difference in the non-AD group (Bonelli *et al.*, 2002). This might be related to the incidence of AD in females being significantly higher than in males (Nussbaum and Ellis, 2003).

SSRIs, other antidepressants, cholinesterase-inhibitors, nootropics, tranquilizers and tramadol did not have an influence on cerebral cell death. Although only the number of SSRIs and other antidepressants were statistically valid, the result is encouraging because no data on antidepressant neurotoxicity are available in the literature that have been obtained *in vitro*. In conclusion, we suggest that typical and atypical neuroleptics should be strictly limited in all elderly patients, especially in females and all patients with AD.

References

Andreassen OA, Jorgensen HA (2000). Neurotoxicity associated with neuroleptic-induced oral dyskinesias in rats. Implications for tardive dyskinesia? *Prog Neurobiol* 61:525–541.

Bailey CD, Graham RM, Nanda N, Davies PJ, Johnson GV (2004). Validity of mouse models for the study of tissue transglutaminase in neurodegenerative diseases. *Mol Cell Neurosci* **25**:493–503.

Behl C, Rupprecht R, Skutella T, Holsboer F (1995). Haloperidol-induced cell death–mechanism and protection with vitamin E in vitro. *Neuroreport* **7**:360–364.

Bonelli RM, Aschoff A, Niederwieser G, Heuberger C, Jirikowski G (2002). Cerebrospinal fluid tissue transglutaminase as a biochemical marker for Alzheimer's disease. *Neurobiol Dis* **11**:106–110.

Cadet JL, Kahler LA (1994). Free radical mechanisms in schizophrenia and tardive dyskinesia. *Neurosci Biobehav Rev* **18**:457–467.

Casey DE (2004). Pathophysiology of antipsychotic drug-induced movement disorders. *J Clin Psychiatry* **65 (suppl 9)**:25–28.

Cummings JL (2004). Alzheimer's disease. *N Engl J Med* **351**:56–67.

Dalgalarrondo P, Gattaz WF (1994). Basal ganglia abnormalities in tardive dyskinesia. Possible relationship with duration of neuroleptic treatment. *Eur Arch Psychiatry Clin Neurosci* **244**:272–277.

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

Gil-Ad I, Shtaif B, Shiloh R, Weizman A (2001). Evaluation of the neurotoxic activity of typical and atypical neuroleptics: relevance to iatrogenic extrapyramidal symptoms. *Cell Mol Neurobiol* **21**:705–716.

Johnson GV, Cox TM, Lockhart JP, Zinnerman MD, Miller ML, Powers RE (1997). Transglutaminase activity is increased in Alzheimer's disease brain. *Brain Res* **751**:323–329.

Lesort M, Tucholski J, Miller ML, Johnson GV (2000). Tissue transglutaminase: a possible role in neurodegenerative diseases. *Prog Neurobiol* **61**: 439–463.

Mion CC, Andreasen NC, Arndt S, Swayze VW II, Cohen GA (1991). MRI abnormalities in tardive dyskinesia. *Psychiatry Res* **40**:157–166.

Noh JS, Kang HJ, Kim EY, Sohn S, Chung YK, Kim SU, Gwag BJ (2000). Haloperidol-induced neuronal apoptosis: role of p38 and c-Jun-NH(2)-terminal protein kinase. *J Neurochem* **75**:2327–2334.

Nussbaum RL, Ellis CE (2003). Alzheimer's disease and Parkinson's disease. *N Engl J Med* **348**:1356–1364.

Oliverio S, Amendola A, Rodolfo C, Spinedi A, Piacentini M (1999). Inhibition of 'tissue' transglutaminase increases cell survival by preventing apoptosis. *J Biol Chem* **274**:34123–34128.

Patt RB, Proper G, Reddy S (1994). The neuroleptics as adjuvant analgesics. *J Pain Symptom Manage* **9**:446–453.

Post A, Rucker M, Ohl F, Uhr M, Holsboer F, Almeida OF, Michaelidis TM (2002). Mechanisms underlying the protective potential of alpha-tocopherol (vitamin E) against haloperidol-associated neurotoxicity. *Neuropsychopharmacology* **26**:397–407.

Sunderland T, Cohen BM (1987). Blood to brain distribution of neuroleptics. *Psychiatry Res* **20**:299–305.

Ukai W, Ozawa H, Tateno M, Hashimoto E, Saito T (2004). Neurotoxic potential of haloperidol in comparison with risperidone: implication of Akt-mediated signal changes by haloperidol. *J Neural Transm* **111**:667–681.

Volokhina EB, Hulshof R, Haanen C, Vermes I (2003). Tissue transglutaminase mRNA expression in apoptotic cell death. *Apoptosis* **8**:673–679.

Yassa R, Jeste DV (1992). Gender differences in tardive dyskinesia: a critical review of the literature. *Schizophr Bull* **18**:701–715.

Yassa R, Nastase C, Dupont D, Thibeau M (1992). Tardive dyskinesia in elderly psychiatric patients: a 5-year study. *Am J Psychiatry* **149**:1206–1211.

Zitman FG, Linssen AC, Edelbroek PM, Van Kempen GM (1991). Does addition of low-dose flupentixol enhance the analgetic effects of low-dose amitriptyline in somatoform pain disorder? *Pain* **47**:25–30.

Zitman FG, Pennings TM, Raes DC, Hekster YA (1994). Neuroleptic drug use in nonpsychiatric departments of a Dutch university hospital. *Gen Hosp Psychiatry* **16**:32–37.