

Full Length Research Paper

Furosemide attenuates ketamine - induced cognitive deficits

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The aim of the study was to investigate the effect of furosemide on the cognitive deficits induced by sub-anaesthetic doses of ketamine in healthy volunteers. It was a prospective, single-blind study in which 50 volunteers between the ages of 18 and 30 years were randomly selected to receive intravenous 0.4mg/kg of ketamine and placebo and another 50 volunteers matched for age and sex were randomly selected to receive intravenous 0.4mg/kg of ketamine and 0.1mg/kg of furosemide. The Mini-Mental Status Examination was used to score the symptoms of cognitive deficits induced by ketamine. The results showed that ketamine administration induces cognitive deficits in human volunteers and the Mini-Mental Status Examination revealed mild impaired cognitive functioning (average rating at 20 minutes of 16-24). Average duration of the cognitive deficits was 52 ± 12.0 minutes for the ketamine-placebo group and was 20 ± 8.5 minutes for the ketamine-furosemide group. The difference in the duration of symptoms between the two groups was statistically significant ($P < 0.01$). In conclusion, the results suggest that furosemide is able to ameliorate the cognitive deficits induced by ketamine.

Keywords: Ketamine, furosemide, cognitive deficits.

INTRODUCTION

The glutamate hypothesis originated in and remains to a considerable extent driven by the effects of N-methyl-D-aspartate (NMDA) receptor antagonists such as ketamine (Pomarol-Clotet et al., 2006).

Ketamine is a dissociative anaesthetic agent capable of producing depersonalization, derealisation and psychogenic amnesia and a non-competitive antagonist of the NMDA receptor. Apart from producing positive and negative symptoms of psychosis similar to those seen in schizophrenia, ketamine induces cognitive impairments such as increased distractibility and decreased verbal fluency similar to the cognitive impairment in schizophrenia (Mechri et al., 2001) who noted the effects to be transitional, reversible and influenced by time. In patients with schizophrenia and healthy volunteers receiving ketamine, scores for the three of the four highest rated items on the thought, language and communication (TLC) scale, i.e. poverty of speech, circumstantiality and loss of goal were the same (Adler et

al., 1999; Krystal et al., 1994). Ketamine has been found to induce a dose-related, short worsening of mental status of schizophrenics (Lahti et al., 1995). It also induces nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase activity which decreases parvalbumin-containing interneurons in the prefrontal cortex leading to impairment in brain circuitry involved in memory and attention which are the hall-marks of schizophrenia (Moran, 2008) who found that ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-oxidase producing superoxide.

Mechanisms of action of furosemide in psychosis

Long-term potentiation is a critical neural process that is likely to underlie learning and memory formation (Thompson, 2000). In schizophrenia, there is evidence that long-term potentiation (LTP) is impaired (Frantseva et al., 2008) who noted that the deficits in learning and memory in schizophrenia may be mediated through the disordered LTP.

Furosemide enhances LTP by blocking isoform 2 of the potassium-chloride co-transporter (KCC2) function,

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thereby inducing EPSP-spike potentiation (Wang et al., 2006) and the impairment of LTP induced by the NMDA receptor antagonist MK-801 (dizocilpine) is significantly reversed by the KCC2 blocker, furosemide (Liu et al., 2009). Depression is a core symptom of schizophrenia (Bleuler, 1924) and there is enhanced expression of the KCC2 cotransporter in spontaneously depressed Flinders Sensitive Line rats (Matrisciano et al., 2010). Additionally, there is altered expression of regulators of the cortical chloride transporters NKCC1 and KCC2 in schizophrenia with increased probability of net excitation by depolarizing GABA (Arion et al., 2011; Kalkman, 2011). Furthermore, the chloride transporter blocker, furosemide may indirectly increase cerebrospinal glutamate levels which is low in the disease by inhibiting the $\text{Ca}^{2+}/\text{Cl}^{-}$ induced increase in L-[^3H] glutamate binding (Recasens et al., 1987 and Pin et al., 1984) and decrease striate body glutamate levels (Kim et al., 1983). Moreover, furosemide may affect ligand-gated chloride channels which may be receptors for dopamine and serotonin (Ringstad et al., 2009 and Vodyanoy et al., 1985). The antagonism of apomorphine by furosemide reported by Vodyanoy et al in 1985 may also be important to elucidate the attenuation of psychosis by furosemide.

Furosemide has been shown capable of antagonizing ketamine-induced generation of superoxide which may be responsible for its effects (Soloperto et al., 1991; Silva et al., 2010).

There may be increased cytosolic calcium in schizophrenics over normal controls (Tan et al., 1995) and furosemide may lower cytosolic calcium (Rubin et al., 1995).

Ketamine enhances GABA currents which might contribute to its sedative effect. Furosemide is able to block the ketamine-induced currents arising from α 6-subunit containing GABA_A receptors.

The aim of the study was to investigate the effect of intravenous furosemide on the cognitive deficits induced by subanaesthetic doses of intravenous ketamine in healthy volunteers, and this was considered worthwhile after our unpublished observation of the beneficial effect of adjunctive use of furosemide in some forms of psychosis.

METHODS

This prospective, single-blind, randomized study was undertaken in the Department of Pharmacology and Therapeutics, A. A. U, Ekpoma and Oseghale Oriaifo Medical Centre, Idumebo-Ekpoma between 2010 and 2011. Two cohorts of 50 healthy volunteers in each group were randomly selected to receive ketamine (NAFDAC no. 04-6318) and placebo, and ketamine and furosemide (NAFDAC No. 04-7546) respectively after a pre-treatment clinical and laboratory work-up which showed them to be

free of cardiovascular, central nervous system, renal, hepatic and pulmonary diseases. They were aged between 18 and 30 years from whom consent was obtained beforehand and project was sanctioned by the college ethical committee. Alcoholics were also excluded from the study.

0.4mg/kg of ketamine was administered with placebo intravenously to one group and 0.4mg/kg of ketamine was administered intravenously to the test group followed by 0.1mg/kg of furosemide in separate syringes. The effects on cognition of ketamine-placebo versus ketamine-furosemide were monitored for 90 minutes recording the time- to- disappear or duration of any cognitive deficit observed. Also, the Mini-Mental Status Examination (MMSE) (Folstein et al., 1975; Harvey et al., 1995) which is similar to the Brief Cognitive Rating Scale (BCRS) was administered to the volunteers and used to rate the level of cognitive impairment.

The Mini-Mental Status Examination (Table 2)

Interpretation

Any score greater than or equal to 25 points (out of 30) is effectively normal (intact). Below this, scores can indicate severe (≤ 9 points), moderate (10-20 points) or mild (21-24 points).

RESULTS

Cognitive deficits induced by ketamine (Table 1)

- 1) Impairment of executive function: Impairment of executive function which co-ordinates cognitive resources may be revealed by disorders in abstract thinking. Abstract thinking and understanding of idioms is impaired by intravenous ketamine administration. It persists for 50 ± 12.0 minutes and this is reduced to 20 ± 13.0 minutes with intravenous furosemide.
- 2) Disorganized thinking: Disorganized thinking is revealed by incoherent or incomprehensible speech and was noticed in these volunteers. It persists for 30 ± 9.0 minutes and was shortened to 10 ± 8.0 minutes by furosemide.
- 3) Poor memory: Ketamine makes the volunteers unable to retain and use information and this lasts for 60 ± 6.0 minutes and was shortened to 20 ± 5.0 minutes by furosemide.
- 4) Poor concentration: Ketamine causes distractible speech and mispronunciation of words which was noticed in these volunteers. It lasted for 50 ± 11.0 minutes which was reduced to 20 ± 12.0 minutes by ketamine
- 5) Difficulty expressing thought: These volunteers under ketamine exhibited poverty of speech manifested by very brief unelaborated replies to questions such as 'it's

Table 2. The mini-mental status examination

Category	Possible points	Description
Orientation to time	5	From broadest to most narrow. Orientation to time has been correlated with future decline.
Orientation to place	5	From broadest to most narrow. This is sometimes narrowed down to streets and sometimes to floor.
Registration	3	Repeating named prompts
Attention and calculation	5	Serial sevens, or spelling "world" backwards It has been suggested that serial sevens may be more appropriate in a population where English is not the first language.
Recall	3	Registration recall
Language	2	Name a pencil and a watch
Repetition	1	Speaking back a phrase
Complex commands	6	Varies. Can involve drawing figure shown.

Table 1. Cognitive Deficits Induced by Ketamine and Effect of Furosemide on Symptom Duration.

Symptom	Duration of symptoms in control group (minutes \pm SD)	Duration of symptoms in volunteers given furosemide (minutes \pm SD)
Aberration in executive functioning	50 \pm 12.0	20 \pm 13.0
Disorganised Thinking	30 \pm 9.0	10 \pm 8.0
Poor memory	60 \pm 6.0	20 \pm 5.0
Poor concentration	50 \pm 11.0	20 \pm 12.0
Difficulty expressing thought	60 \pm 5.0	25 \pm 10.0
Difficulty integrating thought	30 \pm 9.0	10 \pm 6.0
Average	52 \pm 12.0	20 \pm 8.5

Average duration of symptoms was 20 \pm 8.5 minutes in test normal volunteers given ketamine-furosemide and was 52 \pm 12 minutes in control normal volunteers who had only ketamine and placebo. The difference was statistically significant ($P < 0.01$)

okay, it's okay'; and poverty of content of speech manifested by long speech conveying little information. They also exhibited thought block revealed by interruption of a train of speech before idea is completed. Circumstantiality in which speech is delayed in reaching its goal was also exhibited. Perseveration or repetitiousness (Covington et al., 2007) and tangentiality in which replies to questions are irrelevant were also exhibited. This lasted for 60 \pm 5.0 minutes and was reduced to 25 \pm 10.0 minutes with furosemide.

6) Difficulty integrating thought: The volunteers under ketamine also showed illogical reasoning. This lasted for 30 \pm 9.0 minutes and was reduced to 10 \pm 6.0 minutes with furosemide.

The average time of symptoms-duration was 52 \pm 12.0 minutes for the control group and 20 \pm 8.5 minutes for the test group given furosemide. The difference was statistically significant ($P < 0.01$).

MMSE rating

At 20 minutes, the ketamine-placebo group rated an average of 16-24 while the test group who had furosemide rated 23-28. The difference was statistically significant ($P < 0.05$).

DISCUSSION

The study confirms the results of previous investigators that single low-dose of an NMDA receptor antagonist such as ketamine produces schizophrenic-like symptoms in healthy individuals (Adler, 1999 and Lahti et al., 1995). The NMDA receptor has been shown to play a direct role in two types of explicit (episodic and semantic) memory and ketamine has been shown to produce decrements in free recall, recognition memory and attention (Malhotra et al., 1996). Ketamine is now known to produce a formal

thought disorder as well as impairments in working and semantic memory. The degree of ketamine-induced thought disorder significantly correlates with ketamine-induced decreases in working memory (Adler et al., 1998) and may suggest that ketamine-induced deficits in working memory are associated with ketamine-induced thought disorder. A core feature of schizophrenia is the impairment of certain cognitive functions such as working memory that are dependent on the circuitry of the dorsolateral prefrontal cortex (Elvevag and Goldberg, 2000) and there has been a repeated demonstration of a relationship between the severity of neurocognitive deficits and the severity of deficits in functioning in schizophrenic patients (Green, 1996; Andreassen, 2010). Psychotic symptoms of paranoia, perceptual changes, thought disorganization, negativism, apathy, withdrawal, poverty of speech, perseveration and catatonic posturing that are features of schizophrenia (Pomarol-Clotet et al., 2006) are now known to be associated with NMDA antagonists such as ketamine causing decreased glutamatergic function at NMDA receptors (Tsapakis and Travis, 2002) and subanaesthetic doses of ketamine can induce a dose-related, short (< 30 minutes) worsening of mental status in schizophrenia (Lahti et al., 1995).

In this study, ketamine induced a constellation of cognitive dysfunction in the volunteers which included disordered executive function, disorganized thinking, poor memory, poor concentration, difficulty expressing thought and difficulty integrating thought. The results show that the symptom-duration was significantly reduced by furosemide and test volunteers given furosemide significantly rated better on the MMSE.

NMDA hypofunction caused by ketamine up-regulates the dopaminergic system in the mesolimbic dopamine pathway through disinhibition of tonically inhibitory GABA interneurons to cause the positive symptoms of schizophrenia of hallucinations and delusions (Lewis and Gonzalez-Burgos, 2006; Homnynoun and Moghaddam, 2007; Stahl, 2007). Also, NMDA hypofunction induces down-regulation of the meso-cortical dopamine pathway to cause the negative and cognitive symptoms of schizophrenia of affective and memory problems (Stahl, 2007). At the molecular level, this GABAergic disinhibition mediated by NMDA hypofunction impairs long-term potentiation (LTP) and this impairment of LTP has been found to be significantly reversed by furosemide, a KCC2 blocker (Liu et al., 2009). It has been suggested (Frantseva et al., 2008) that cognitive impairments in schizophrenia might be due to a disordered LTP. Additionally, persistent increase in NADPH oxidase activity by ketamine occasions more superoxide production and this decreases parvalbumin expression and GABA-producing enzyme (GAD 67) by interneurons in prefrontal cortex leading to impairment in brain circuitry involved in memory and attention (Behrens et al., 2007). It is already known that GAD 67 is down-regulated in schizophrenics who may express GABAergic deficit-

related alterations of pyramidal neural function (Costa et al., 2004). Furosemide is able to inhibit superoxide generation (Silva et al., 2010; Soloperto et al., 1991) which may partly account for its attenuation of ketamine-induced behavioral alterations and cognitive decline observed in this study and which has not been reported previously; while furosemide has been reported not to affect the elimination of ketamine (Hanna et al., 1988).

Hevers et al., (2008) reported that furosemide blocked the ketamine-induced currents arising from $\alpha 6$ -containing GABA_A receptors which may partly explain furosemide's antagonism of ketamine's sedative effects. Also, the blocking of the $\text{Ca}^{2+}/\text{Cl}^{-}$ -dependent excitatory amino acid transporter (EAAT) by furosemide (Pin et al., 1984) may prevent reuptake of glutamate into membrane vesicles and help to offset glutamate hypofunction caused by NMDA receptor antagonists.

Moreover, studies by Toyooka et al (2002) showed that there is impaired release of brain-derived neurotrophic factor (BDNF) from platelets in schizophrenia. Furosemide may offset this since it has been shown able to increase the angiotensin 11-induced expression of BDNF from human and rat adrenocortical cells (Szekeres et al., 2010).

In conclusion, intravenous administration of the dissociative agent, ketamine, induces thought and cognitive disorders in healthy volunteers. These thought and cognitive disorders were significantly attenuated by furosemide.

REFERENCES

- Andreassen NC (2010). Cognitive deficits in schizophrenia. *Schizophrenia bulletin*. Oxfordjournals.org
- Arion D, Lewis DA (2011). Altered expression of regulators of the cortical chloride cotransporters NKCC1 and KCC2 in schizophrenia. *Arch. Gen. Psychiatry*. 68(1): 21-31
- Adler CM, Malhotra AK, Elman I, Goldberg T, Egan M, Pickar D, Breier A (1999). Comparison of ketamine-induced thought disorder in healthy volunteers and thought disorder in schizophrenia. *Am.J.Psychiatry*. 156(10):1646-9.
- Adler CM, Goldberg TE, Malhotra A, Pickar D, Breier A (1998). Effects of ketamine on thought disorder, working memory and semantic memory in healthy volunteers. *Biological Psychiatry*. 43 (11):811-816.
- Behrens MG, Ali SS, Dao DN, Lucero J, Shekhtman G, Quick KL, Dugan LL (2007). Ketamine-induced loss of phenotype of fast-spiking interneurons is mediated by NADPH-Oxidase. *Science*. 318 (5856):1645-1652
- Bleuler E (1924). *Textbook of Psychiatry*. English edition by AA Brill; New York
- Costa E, Davis JM, Dong E, Grayson DR, Guidotti A, Tremolizzo L, Veldic M (2004). A GABAergic cortical deficit dominates schizophrenia pathophysiology. *Crit. Rev Neurobiol*. 16: 1-23
- Covington MA, Reidel WJ, Brown C, He C, Morris E, Weinstein S (2004). Does ketamine mimic aspects of schizophrenic speech? *J. Psychopharmacol*. 21 (3):338-346
- Elvevag B, Goldberg TE (2000). Cognitive impairment in schizophrenia is the core of the disorder. *Crit Rev Neurobiol*. 14(1): 1-21
- Frantseva MV, Fitzgerald PB, Chen R, Moller B, Daigle M, Daskalakis ZJ (2008). Evidence for impaired long-term potentiation in schizophrenia and its relationship to motor skill learning. *CerebralCortex*. 18 (5):990-996.
- Folstein MF, Folstein SE, McHugh PR (1975). Mini-Mental State, a

- practical method for grading the cognitive state of patients for the clinician. *J. Psychiatric Res.* 12:189-198.
- Green MF (1996). What are the functional consequences of neurocognitive deficits in schizophrenia? *Am J. Psychiatry.* 15:321-330.
- Hanna RM, Borchard RE, Schmidt SL (1988). Effect of diuretics on ketamine and sulfanilate elimination in cats. *J. Vet. Pharmacol Ther.* 11(2):121-9
- Homnyouon H, Moghaddam B (2007). NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *The J. Neuroscience.* 27(43):11496-11500.
- Harvey PD, White L, Parrella M, et al., (1995). The longitudinal stability of cognitive impairment in schizophrenia. Mini-Mental State Scores at one-and two-year follow-ups in geriatric in-patients. *British J. Psychiatry.* 166: 630-633.
- Hevers W, Hadley SH, Luddeno H, Amin J (2008). Ketamine, but not phencyclidine, selectively modulates cerebellar GABA_A receptors containing $\alpha 6$ and δ subunits. *J. Neuroscience.* 28(20): 5383-5393
- Krystal JH, Karpu LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, Heninger GR, Bowers MB Jr, Charney DS (1994). Subanaesthetic effects of the non-competitive NMDA antagonist ketamine in humans: Psychotomimetic, perceptual, cognitive and neuroendocrine responses. *Arch. Gen. Psych.* 51 (3):199-244.
- Kalkman HO. Alterations in the expression of neuronal chloride transporters may contribute to schizophrenia. *Progress in Neuro-Psychopharmacology and Biological Psychiatry.* 2011; 36(2): 410-414
- Kim JS, Kornhuber HH, Holzmüller B, Schmid-Burgk W, Mergner T, Krzepinski G (1980). Reduction of cerebrospinal fluid glutamic acid in Huntington's chorea and in schizophrenia patients. *Arch. Psychiatr. Nervenkr.* 228:7-10.
- Kim JJ, Claus D, Kornhuber HH (1983). Cerebral glutamate, neuroleptic drugs and schizophrenia: Increase of cerebrospinal fluid glutamate levels and decrease of striate body glutamate levels following sulphuride treatment in rats. *European Neurology.* 22: 367-370.
- Lahti AC, Koffel D, LaPorte D, Tamminga CA (1995). Subanaesthetic doses of ketamine stimulate psychosis in schizophrenia. *Neuropsychopharmacology.* 13:9-19.
- Liu Y, Chen J, Song T, Hu C, Tang Y, Zhang X, Zhao J (2009). Contribution of K⁺Cl⁻ cotransporter2 in MK-801-induced impairment of long-term potentiation. *Behav Brain Res.* 201(2):300-4.
- Lewis DA, Gonzalez-Burgos G (2006). Pathophysiologically based treatment interventions in schizophrenia. *Nat Med.* 12:1016-1022.
- Malhotra AK, Pinals DA, Weingartner H, Sirocco K, Missar D, Pickar D, Breier A (1996). NMDA receptor function and human cognition: The effects of ketamine in healthy volunteers. *Neuropsychopharmacology.* 14: 301-307.
- Moran M (2008). Ketamine findings suggest path to new schizophrenia drugs. *Psychiatric News.* 43(2): 34.
- Matrisciano F, Nasca C, Molinaro G, Rizzo B, Scaccianoce S, Raggi MA, Mercolini L, Biagioni F, Mathè AA, Sanna E, Maciocco E, Pignatelli M, Biggio G, Nicoletti F (2010). Enhanced expression of the neuronal K/Cl cotransporter, KCC2, in spontaneously depressed Flinders Sensitive Line rats. *Brain Research.* 1325: 112-120.
- Mechri A, Saoud M, Khiari G, d'Amato T, Dalery J, Gaha L (2001). Glutaminergic hypothesis of schizophrenia: clinical research studies with ketamine. *Encephale.* 27 (1):53-9.
- Pomaro-Clotet E, GD Honey, GK Murray, PR Corlett, AR Absalom, M Lee, PJ McKenna, ET Bullmore, PC Fletcher (2006). Psychological effects of ketamine in healthy volunteers. *Phenomenological Study. British J. Psychiatry.* 189: 173-179
- Pin JP, Bockaert J, Recasens M (1984). The Ca²⁺/Cl⁻ dependent L-[³H]glutamate binding: a new receptor or a particular transport process? *FEBS Letters.* 175 (1): 31-36.
- Ringstad N, Abe N, Horvitz HR (2009). Ligand-gated chloride channels are receptors for biogenic amines in *C. elegans*. *Science.* 325 (5936):96-100.
- Recasens M, Pin JP, Bockaert J (1987). Chloride transport blockers inhibit the chloride-dependent glutamate binding to rat brain membranes. *Neuroscience Letters.* 74:211-216.
- Rubin Y, Kessler-Icekson G, Navon G (1995). The effect of furosemide on calcium ion concentration in myocardial cells. *Cell Calcium.* 18(2):135-139.
- Stahl SM (2007). Beyond the dopamine hypothesis to the NMDA glutamate receptor hypofunction hypothesis of schizophrenia. *CNS Spectrum.* 12 (4):265-268.
- Szekeres M, Nadasy GL, Turu G, Supeki K, Svidonya L, Buday L, Chaplin T, Clark A, Hunyady T (2010). Angiotensin 11-induced expression of BDNF in human and rat adrenocortical cells. *Endocrinology.* 151(4): 1695-1703.
- Silva GB, Garvin JL (2010). Rac 1 mediates NaCl-induced superoxide generation in thick ascending limb. *Am. J. Physiol. Res.* 298(2): F421-5.
- Soloperto M, Marini M, Brasca C, Fasoli A, Mattoli S (1991). The protective effect of furosemide on the generation of superoxide anions by human bronchial epithelial cells and pulmonary macrophages in vitro. *Pulm Pharmacol.* 4(2):80-4.
- Tan CH, Lee HS, Kua EH, Peh LH (1995). Resting and thrombin-stimulated cytosolic calcium in platelets with alcoholic withdrawal, bipolar manic disorder and chronic schizophrenia. *Life Sci.* 56(21):1817-23
- Tsappakis EM, Travis MJ (2002). Glutamate and psychiatric disorders. *Advances in psychiatric treatment.* 8:189-197.
- Toyooka K, Asama K, Watanabe Y, Muratake T, Takahashi M, Someya T, Nawa H (2002). Decreased levels of brain-derived neurotrophic factors in serum of chronic schizophrenic patients. *Psychiatry Res.* 110:249-257
- Thompson SM (2000). Cognition. Long-Term Potentiation. *Am. J. Psychiatry.* 157:492
- Vodyanoy V, Vodyanoy I, Murphy RB (1985). Furosemide blocks the apomorphine-elicited Cl⁻ channel activity of rat striatal dopamine receptors functionally reconstituted into bimolecular lipid membrane. *Neuroscience Letters.* 62(1): 103-106.
- Wang W, Gong N, Xu TL (2006). Down-regulation of KCC2 following Long-Term Potentiation contributes to EPSP-Spike potentiation in rat hippocampus. *Biochem Biophys Res Commun.* 343(4): 1209-15.