Olanzapine Does not Aggrevate Ischemic Neuronal Injury by Focal Cerebral Ischemia: A Dose Related Restriction of the Neuroprotective Effect?

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Abstract: We have previously shown the neuroprotective effect of atypical antipsychotic agents by experimental cerebral ischemia. However the impact of their high dose related side effects on their low dosage related neuroprotectivity is still unknown.We evaluated the possible neuroprotective effects of high dose olanzapine (10mg/kg) treatment on ischemic brain injury 24 hr after permanent cerebral ischemia. Olanzapine showed neither a neuroprotective nor a neurotoxic effect after focal cerebral ischemia. This finding could suggest that dose related side effect of olanzapine could involve a restriction of its neuroprotective effect unlike lower doses that have been reported to have neuroprotective effect.

Key Words: Stroke, atypical antipsychotics, mood disorders.

INTRODUCTION

Atypical antipsychotic agents are discovered to have some beneficial effects beyond their effectiveness as antipsychotic drugs. Among these initially unexpected effects are their potential effects as mood stabilizers by bipolar disorder and their efficacy in improving long term outcome in schizophrenia [1-5]. In regard of their role in poststroke depression which affects approximately 20% to 40 % of all poststroke patients [6, 7], we have previously shown the neuroprotective effect of risperidone and olanzapine after cerebral ischemia in spesific dose ranges [8,9]. This was suggesting other studies indicating the neuroprotective role of these agents in various experimental models [10-16]. But it is still unclear whether their high dose related side effects could involve a possible neurotoxicity which can lead to a restriction of their neuroprotective effect. To examine this matter we evaluated the neuroprotective effect of olanzapine after permanent focal cerebral ischemia at its high dose and compared it with the sham operated group.

MATERIAL AND METHODS

Experimental Groups

All experimental procedures were carried out with governmental approval according to local guidelines for the care and use of laboratory animals. Adult male C57BL/6j mice weighing 21-25g was assigned to the following experiments and groups: I. Intraperitoneal administration of (a) 10 mg/kg olanzapine (n =8 animals) and (b) 0.2 ml vehicle (% 0.09 NaCl) (n=8 animals), starting at the onset of 24 hours of permanent focal cerebral ischemia. Olanzapine (Lilly Chemical Pharmaceuticals) dissolved in 0.1N HCl and then buffered with NaOH (final pH=7.1). By referring to the 70-100 times higher metabolic rate in mice we determined the averaged high doses of olanzapine as 10 mg/kg [17].

Induction of Ischemia

Animals were anesthetized with 1% halothane (30% O2, remainder N2O). Rectal temperature was maintained between 36.5 and 37.0 °C using a feedback-controlled heating system. During the experiments, cerebral blood flow was measured by laser Doppler flowmetry (LDF) using a flexible 0.5 mm fiber optic probe (Perimed, Stockholm, Sweden), which was attached to the intact skull overlying the MCA territory (2 mm posterior / 6 mm lateral from bregma). LDF changes were monitored up to 90 min after the onset of ischemia.

Focal cerebral ischemia was induced using an intraluminal filament technique [8]. A midline neck incision was made, and the left common and external carotid arteries were isolated and ligated. A microvascular clip (FE691, Aesculap, Tuttlingen, Germany) was temporarily placed on the internal carotid artery. A 8-0 nylon monofilament (Ethilon; Ethicon, Norderstedt, Germany) coated with silicon resin (Xantopren, Bayer Dental, Osaka, Japan; diameter of the coated thread: 190-200 μm) was introduced through a small incision into the common carotid artery and advanced 9 mm distal to the carotid bifurcation for permanent occlusion of the MCA. Anesthesia was discontinued and animals were placed into their home cages.

Triphenyltetrazolium Chloride (TTC) Staining

Animals were reanesthetized with overdose halothane and decapitated. Brains were incubated for 5 min in ice-cold isotonic saline and coronally cut into five 2 mm slices using a mouse brain matrix (BRM-2000C; Activational System Inc., MI, USA). These slices were immediately stained with 2% 2,3,5- triphenyltetrazolium chloride (TTC) for 20 minutes. The border between infarcted and non-infarcted tissue was outlined using an image analysis system, and the area of in-

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farction was measured by subtracting the area of the non-lesioned ipsilateral hemisphere from that of the contralateral side. The infarct volume was calculated by integration of the lesion areas [8].

Statistics

All values are given as mean ± S.D. Differences between groups were compared by using oneway ANOVA analysis followed by LSD tests after permanent cerebral ischemia. P values < 0.05 were considered to indicate statistical significance.

RESULTS

Laser Doppler Flow (LDF)

Mean LDF reproducibly declined to <25% of pre-ischemic control levels immediately after thread insertion in all animal groups. No differences were seen between various animal groups (Fig. **2**).

Triphenyltetrazolium Chloride (TTC) Staining: Infarct Size

Reproducible brain infarcts were obtained in the 10 mg/kg of olanzapine or 0.2 ml of vehicle treated groups (Fig. **1**). Intraperitoneal administration of 10 mg/kg olanzapine was found neither neuroprotective nor neurotoxic.

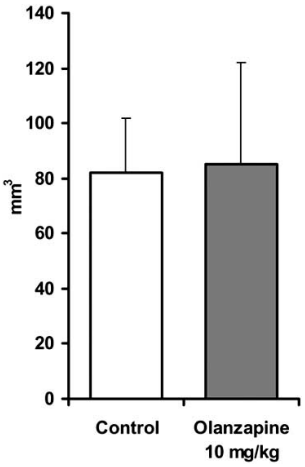

Fig. (1). Infarct volumes of mice subjected to permanent cerebral ischemia. Animals treated with olanzapine (10 mg/kg). Note that 10 mg/kg olanzapine was not neuroprotective or neurotoxic (p>0.05).

DISCUSSION

Atypical antipsychotic drugs offer several notable benefits over typical antipsychotics, including greater improvement in negative symptoms, cognitive function, prevention of deterioration, and quality of life, and fewer extrapyramidal symptoms (EPS) [18].

However, concerns about EPS have been replaced by concerns about other side effects which can be partly the potential contribution of different receptors to metabolic and/or neurologic side effects associated with atypical antipsychotic treatment [18].

In the ischemic brain it is well known that a massive release of dopamine can amplify the neuronal damage caused by excitotoxicity and energy deprivation [19-21]. Besides dopamin, serotonin is also shown to modulate the postsynaptic effects of glutamate and leads to reduction of blood flow during cerebral ischemia [22,23]. Therefore, ischemia-induced abnormal release of such neurotransmitters in vulnerable brain areas may represent a critical factor that transforms transient ischemic attack into an ischemic episode resulting in irreversible consequences in brain tissue and its synaptic circuits.

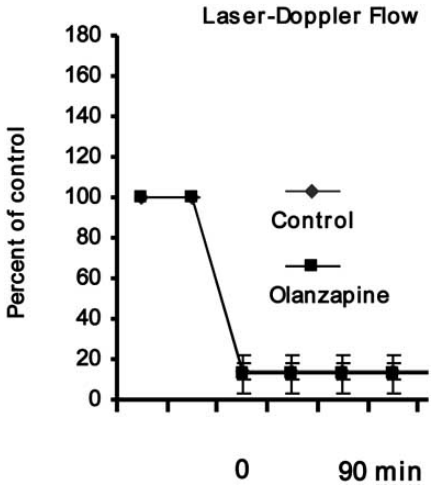

Fig. (2). Laser Doppler flow measurements (LDF) above middle cerebral artery territory during permanent intraluminal thread occlusion in animals treated with vehicle solution or olanzapine. No differences in LDF values were detected between animal groups. Values are means ± S.D.

Olanzapine is one of the member of the atypical neuroleptic drugs and a candidate as a clinically applicable neuroprotective drug. In addition to its well-known high affinity for dopamine (D_1, D_2, $D_{4)}$ and serotonin ($5HT_{2A}$, $5HT_{2C}$, $5HT_3$) receptors, olanzapine is also shown to increase the number of newborn cells in the dentate gyrus and in the prelimbic cortex of the hippocampus [14-16]. However, it has also been reported that olanzapine increases the potent GABA(A) receptor modulator allopregnanolone, which is recently proven to be neuroprotective *in vitro* in cerebral ischemia [24,25].

Although thus far the localization of the 5-HT receptor subtypes in the brain is largely unknown and further studies are ongoing to better define their specific function [26], current data indicate to the neuroprotective role of 5-HT2B receptors through their anti-inflammatory and antiapoptotic protective actions [27]. However the evidences indicating to the neuroprotecive role of 5-hydroxytryptamine ($5\text{-}HT_{2A}$) receptor antagonists as an inhibitor of platelet aggregation and vasoconstriction that is induced by 5-HT [28-31] are also rapidly replicating. Considering the high ($5\text{-}HT_{2A}$) receptor antagonist activity of olanzapine it is of wonder that olanzapine could exert its neuroprotective activity partly through the $5\text{-}HT_{2A}$ receptors in the brain. However the neuroprotective role of $5\text{-}HT_3$ antagonists by experimental cerebral ischemia is still controversial [32-35].

We observed that acute administration of 10 mg/kg of olanzapine does not lead to decrease in infarct size (P>0.05,

ANOVA analysis followed by LSD tests) (Fig. **1**). In compared to our previous study which indicated to a significant neuroprotective effect of olanzapine at lower doses, this findings showed that olanzapine was failed to maintain its neuroprotectivity at higher doses whereas it was also not found neurotoxic. This is interesting in light of recent clinical evidence of an increased rate of cerebrovascular events in olanzapine treated dementia patients, and suggests the safety usage of antipychotics even in the elderly population [36]. Additionally, because olanzapine provided no neurotoxicity even at its higher doses in mice, this study suggests that it might trigger other mechanism(s), including vascular pathology, known to be associated with higher stroke risk in demented patients [37].

It is difficult to estimate what caused the decreasement of the neuroprotectivity of olanzapine without getting the results from furher experiments on the antiapoptotic effects of various atypical antipsychotic agents with different receptor affinities, but it may possible that higher affinity of olanzapine for 5HT2A receptors may carry seratonergic/dopaminergic balance in the brain beyond optimum for prevention of ischemia at higher doses. However, it can be also hypothesized that the anticholinergic activity which increases with the higher doses of olanzapine could have restricted the neuroprotective activity of olanzapine. This is suggesting the data indicating enhancing effect of cholinergic activity in neuroprotection as well as reversal of such effect under cholinergic blockage [38-41]. But it should also not to be forgotten that the failure of neuroprotection could be related to the limited size of penumbra which could have restricted the additive neuroprotective effect olanzapine at its higher doses [42].

In summary, beyond suggesting the safety usage of olanzapine at higher doses by elderly population, this study show that olanzapine provides no additional neuroprotective effect at its higher doses and provides evidence that the restriction of the neuroprotective effect of olanzapine can be an important sign of its high dose related metabolic and/or neurologic side effect. Further experiments to evaluate the long-term clinical reflections of such neuroprotective-toxic interactions of atypical neuroleptics *via* magnetic resonance imaging and spectroscopy studies would be the logical future steps to be taken in the field of psychiatric research.

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