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Pharmacological characterisation of EVT 101, a novel potent and orally acting NR2B subtype selective NMDA antagonist

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Introduction

The glutamate gated NMDA ion channels play key roles in excitatory synaptic transmission (Kemp & McKernan, 2002). NMDA receptors are association with numerous neurological disorders, and, thus, NMDA receptor antagonists, particularly those selectively targeting the NR2B subunit, are of considerable therapeutic interest for several indications, including Alzheimer's disease, pain and depression (Preskorn et al, 2008). Whereas non-selective NMDA receptor antagonists have a narrow therapeutic window between their therapeutic action and mechanism based side-effects, both pre-clinical as well as initial clinical experience indicate that NR2B subtype-selective NMDA antagonists are better tolerated and do not produce the profound CNS adverse effects typical of non-selective NMDA blockers. While several NR2B antagonists have been developed, most of them have suffered from lack of oral bioavailability or off-target effects which have precluded their development. In this report, we describe the *in vitro* and *in vivo* properties of EVT 101, a potent and orally bioavailable NR2B receptor antagonist currently under clinical development for treatment-resistant depression.

Methods

***In vitro* studies**

Binding. [^{3}H]MK-801 and [^{3}H]Ro 25-6981 binding to rat brain membranes was performed as described in Gill et al., 2001, and Mutel et al., 1998.

Electophysiology. CHO-K1 cells were transiently transfected with plasmid containing the cDNA clones encoding the human NR1 subunit of the NMDA receptor (GRIN1) and with plasmids encoding the NR2A (GRIN2A) or NR2B (GRIN2B) subunit. Patch-clamp experiments were performed in the voltage-clamp mode and whole-cell currents recorded. Currents were elicited by application of 100 µM NMDA and 30 µM glycine.

***In vivo* studies**

***Ex vivo* binding:** NR2B receptor occupancy was determined in mice after oral adminitation of EVT 101 by ex vivo binding after i.v. administration of [^{3}H]MK-801 as described by Murray et al., 2000.

Protection from NMDA-induced seizures. EVT 101 was administered to mice 15 min (i.v) and 30 min (p.o.) administration, respectively, before i.c.v. injection of NMDA (0.15 µg/2 µl, Gill et al., 2002) and animal observed for 5 min.

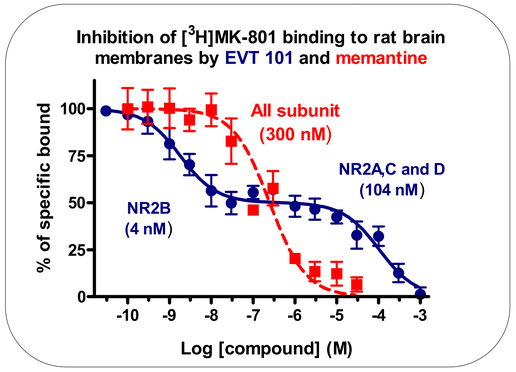
Motor Coordination and locomotor activity. Motor coordination was studied in mice using a revolving Rotarod apparatus. EVT 101 was given i.v. or p.o. 15 or 30 min, respectively, before the test and the latency time to fall off the Rotarod was determined. Locomotor activity was studies in rats over a 4h period after p.o. administration of EVT 101 (Gill et al., 2002).

ADME

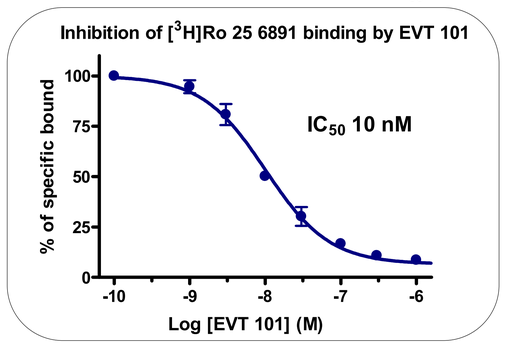
ADME *in vitro* and *in vivo* experiments were performed according to internal Standard Operating Procedures

Results

[^{3}H]MK-801 binding. EVT 101 inhibited [^{3}H]MK-801 binding to rat brain membranes in a biphasic manner with IC_{50}s for the high and low affinity components of 4.4 nM and 104 µM, respectively. In line with previous reports obtained with other NR2B selective antagonists and data obtained in NR2A$^{-/-}$ mice, the high affinity component corresponds to binding of EVT 101 to NR2B subunit containing receptors, whereas the low affinity one corresponds to binding to other NR2 subunits, mostly NR2A. For comparison, inhibition of [^{3}H]MK-801 binding by the non-selective antagonist memantine showed a single component with an IC_{50} of 0.3 µM.



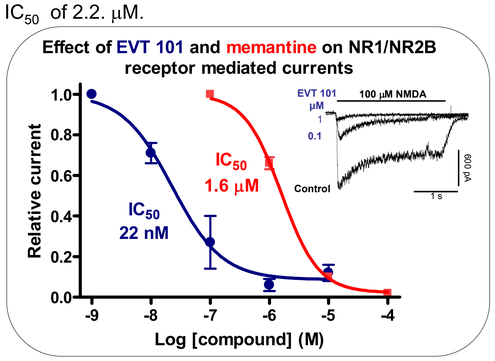
[^{3}H]Ro 25-6981 Binding. Using [^{3}H]Ro 25-6981 to selectively label NR2B subunit-containing NMDA receptors, EVT 101 inhibited the binding of the radioligand to rat brain membranes with an IC_{50} of 10nM.



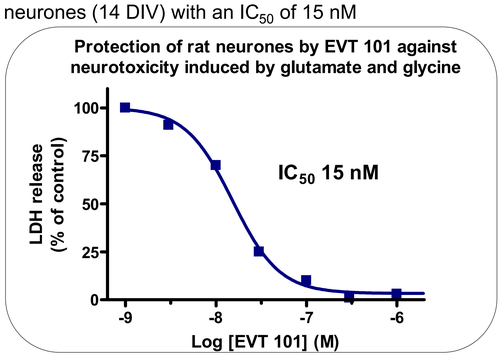
EVT 101 specificity: EVT 101 (up to 10 µM) showed little or no activity at numerous receptors (e.g. muscarinic and aminergic receptors), ion channels and enzymes, thereby indicating a lack of relevant off-target activity.

hERG inhibition: In patch-clamp experiments, EVT 101 inhibited hERG currents with an IC_{50} of 10 µM, a concentration about 1000-times higher than its affinity for NR2B receptors, and >100-fold higher than free plasma concentrations considered therapeutically relevant (Redfern et al., 2002).

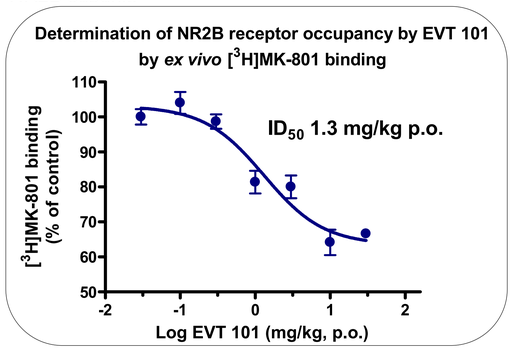
Electophysiology: EVT 101 inhibited hNR1+NR2B receptor mediated currents with an IC_{50} of 22 nM, (nH=1) and an I_{max} of 92%. It had no effect on hNR1/NR2A mediated responses up to the maximal concentration tested of 10 µM. In comparison, memantine was ~100-fold less potent on NR2B subunit containing receptors and inhibited NR1/NR2A with a IC_{50} of 2.2. µM.



Glutamate excitoxicity: EVT 101 inhibited glutamate/glycine-induced toxicity of rat cultured cortical neurones (14 DIV) with an IC_{50} of 15 nM



Determination of NR2B receptor occupancy: Oral administration of EVT 101, 45 min before i.v. administration of [^{3}H]MK-801, resulted in a dose-dependent inhibition of radioligand binding in the brain with an ID_{50} of 1.3 mg/kg. Maximal receptor occupancy by EVT 101 was approximately 40%, corresponding to the fraction of total NMDA receptors containing the NR2B subunit.



Effect of EVT 101 on NMDA-induced Seizures in Mice. EVT 101 dose-dependently antagonized seizures induced in mice by i.c.v. administration of NMDA with similar ED_{50} values after i.v. or p.o administration. In contrast to non-selective NMDA antagonists, even supramaximal anticonvulsant doses of EVT 101 did not impair locomotor activity (rotarod performance).

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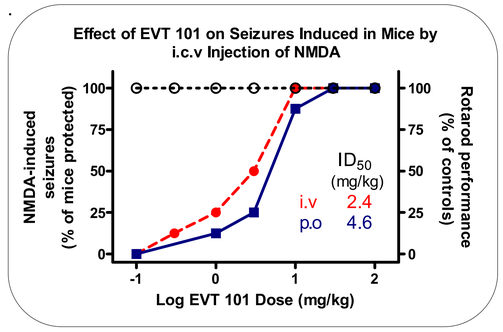
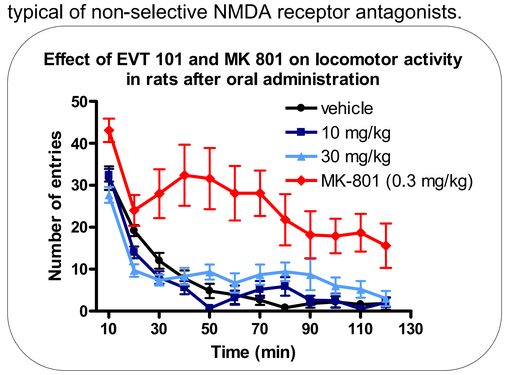
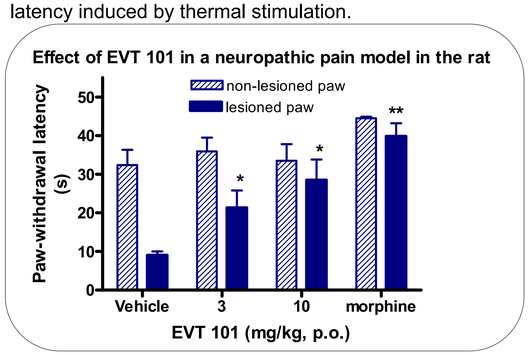


Effect of EVT 101 on Locomotor Activity in Rats. Oral administration of EVT 101 up to 30 mg/kg produced only a marginal increase of locomotor activity which was considerably lower than that observed with low doses of MK-801. Furthermore, EVT 101 did not produce stereotypies, typical of non-selective antagonists. These data indicate that EVT 101 has a much reduced, if any, liability to produce undesired psychostimulant effects typical of non-selective NMDA receptor antagonists.



Effect of EVT 101 in a pain. In a sciatic nerve ligation neuropathic pain model EVT increased paw-withdrawal latency induced by thermal stimulation.



ADME properties

In rats, EVT 101 was rapidly and extensively absorbed after oral administration with a high oral bioavailability (F >100%). Clearance after i.v. and p.o. was 24 and 19 mL/min/kg respectively with a terminal half-life ($t_{1/2}$) of 1.8 (i.v.) and 3.2 h (p.o.).

PK parameters of EVT 101 after oral administration to various species including humans

Species	Dose (mg/kg or mg*)	C_{max} (ng/mL)	AUC_{0-inf} (ng h/mL)	$T_{1/2}$ (h)
Rat	10	1330	9091	3.2
Dog	4	825	1809	1.2
Monkey	3	962	2427	5.3
Human	15*	131	1115	11.9

EVT 101 readily penetrates into the brain, showing in rats a brain/plasma and a CSF/plasma ratio of 0.6 and 0.25 after 5 h i.v. infusion. The brain/plasma and CSF/plasma ratios measured in monkeys were 1 and 0.38.

EVT 101 is not a P-gp substrate and in agreement with its high passive permeability, its CSF concentrations closely approximate free plasma concentrations (32% unbound in humans).

Inhibition of CYP450s in human microsomes

	3A4	2C9	1A2	2D6	2C19
IC_{50} (µM)	>25	>25	>25	11.4	19.3

In human hepatocytes EVT 101 did not induce CYP1A and CYP3A4.

In vitro EVT 101 was metabolised by CYP2C19, CYP3A4 and to a lesser extent by CYP2D6.

Overall, these data indicate a low liability of potential drug-drug interactions.

Conclusions

1. EVT 101 is a potent antagonist at NMDA receptors containing the NR2B subunit with no significant off-target activities
2. EVT 101 is orally active in *in vivo* models without inducing effects on motor coordination or activity typical of non-selective NMDA receptors antagonists
3. EVT 101 has good PK properties and oral availability making it suitable for once-a-day oral dosing in humans
4. EVT 101 has succesfully completed extensive toxicology studies and PhI clinical trials and is currently in a PhIIa proof-of-concept trial in treatment-resistent depression

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