Amino Acids (2000) 19: 167–172

Amino
Acids

NMDA channel blockers: memantine and amino-aklylcyclohexanes – *In vivo* characterization

Review Article

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Accepted September 20, 1999

Summary. The previous overviews provided the basis for better therapeutic efficacy/tolerability of low to moderate affinity NMDA channel blockers. This prediction finds support in in vitro studies comparing protective and plasticity impairing effects (therapeutic vs. side-effect) of memantine and (+)MK-801. In fact it turned out that memantine had a far better therapeutic index. But can it be confirmed in the in vivo situation?

Keywords: Amino acids – NMDA – Channel blockers – Memantine

An important element of any in vivo experiments with CNS active substances is predetermination of some pharmacokinetic aspects such as the relation between dose, serum levels and free brain concentration. Such comparison allows the establishment of serum levels achieved at doses effective in animal models and free brain levels. The former then makes selection of dosing in phase I clinical studies much easier. On the other hand the free brain levels give hints whether the presumed mechanism of action – found through in vitro studies – at the doses used can be verified. A very important and often ignored issue in the use of NMDA channel blockers is the fact that the assessment in brain homogenates might be misleading resulting in over-estimation (15–45 fold) of extracellular brain levels due to accumulation in intracellular compartments (Hesselink et al., 1999). Bearing that in mind we performed brain microdialysis experiments with in vivo recovery to asses ECF levels of memantine and our new lead compound MRZ 2/579. Memantine at the dose of 20 mg/kg produces 1.5 μM concentrations in the brain while MRZ 2/579 at 5 mg/kg leads to 0.8 μM ECF levels. Interestingly, much lower doses of both agents were neuroprotective against NMDA-induced lesion of the NBM i.e. memantine with ED_{50} = 2.8 mg/kg while MRZ 2/579 was already protective at 1 mg/kg (Wenk et al., 1998). Interestingly, when this neuropro-

tective potency and memory impairing effects (passive avoidance) are compared between (+)MK-801 and memantine, it turns out that memantine has over 5 times higher therapeutic window (Parsons et al., 1999). For MRZ 2/579 a similar ratio was found.

Much higher doses of MRZ 2/579 are needed to provide neuroprotection in a transient model of MCA occlusion. In this case bolus of 6 mg/kg followed by infusion of 6 mg/kg/hr leading to 7 μM levels in the ECF and 10 μM in plasma, provides significant protection (over 50%) in the cortex but not striatum (Sopala et al., 1998). Similarly, memantine provides neuroprotection in this model after single injection of 20 mg/kg (Chen et al., 1998).

Neuroprotection is probably one of the most frequent studied actions of NMDA receptor antagonists, however these agents show positive effects in a number of animals models of other neurological/psychiatric disorders (Parsons et al., 1998). Recently, we devoted a lot of attention to the combined use of NMDA receptor antagonists with morphine. It turned out that both memantine and MRZ 2/579 inhibit morphine self administration in mice at very low doses (starting at 1–3 mg/kg) (Semenova et al., 1999). Unexpectedly, (+)MK-801 was ineffective at 0.1 mg/kg providing an example of an qualitative difference between low and high affinity NMDA channel blockers.

Similarly, both memantine and MRZ 2/579 attenuated morphine place preference (Popik and Danysz, 1997; Popik et al., 2000) supporting their antiabuse potential in morphine addicts. This finds confirmation in recent clinical studies showing that memantine attenuates morphine consumption in addicted subjects (Bisaga et al., 1998).

Many studies also suggest that NMDA receptor antagonists inhibit tolerance to the analgesic action of this opioid this also holds true for memantine and MRZ 2/579 (Popik et al., 2000). Even more important was the observation that both agents attenuated morphine tolerance both when morphine treatment was terminated before memantine/MRZ 2/579 treatment started (enhanced extinction) and also when morphine treatment continued (reversal, ibid.).

The above cited studies were based on repetitive treatment with both morphine and NMDA antagonists. A different approach was used by (Houghton et al., 1999) who found that continuous sc. co-infusion of morphine and MRZ 2/579 leads to substantial inhibition of tolerance to the analgesic effects as compared to rats treated with morphine and saline. Generally, a similar profile has been described for (+)MK-801 (Herman et al., 1995).

Tolerance to morphine, and likely to other drugs can be viewed as a kind of synaptic plasticity. NMDA receptors have been implicated in various plasticity processes, hence the fact that NMDA receptor antagonists inhibit tolerance is not unexpected. A similar type of plasticity could play a role in the induction of hyperalgesia (allodynia) in chronic pain state. MRZ 2/579 at low doses (2–5–5 mg/kg) attenuated thermal hyperalgesia in rats produced by carrageenan injection into the paw (Houghton et al., 1999) while memantine was active at 10–15 mg/kg (Eisenberg et al., 1995).

Alcohol has inhibitory effects on NMDA receptor function at concentrations known to be present in alcoholics (Little, 1991). In line with this NMDA receptor antagonists (including memantine and MRZ 2/579) generalize to alcohol in drug discrimination studies in low – therapeutically relevant doses (Faingold et al., 1998; Hölter et al., 2000). In operant conditioning studies responding to obtain alcohol is inhibited by MRZ 2/579 at the dose of 5 mg/kg (Bienkowski et al., 1999) while no selective effect for memantine could be found in the same model (Piasecki et al., 1998). Rats with long lasting (several month) experience with alcohol show relapse behavior upon withdrawal. This relapse drinking is selectively inhibited by continuos sc. infusion of memantine and MRZ 2/579 (Hölter et al., 1996; Hölter et al., 2000).

Similarly to memantine, MRZ 2/579 shows antiparkinsonian like activity in animal models such as haloperidol-induced catalepsy, reserpine-induced sedation and rotation in rats with unilateral lesion to the nigro-striatal system (Danysz et al., 1994; Karcz-Kubicha et al., 1999). However, effective doses (except for the first model) are above the predicted therapeutically relevant range and in none of these models was efficacy better than following (+)MK-801 treatment. Synergism with L-DOPA was obtained at somewhat lower doses (ibid.).

Some experimental evidence suggests that NMDA receptor antagonism may be a valid approach in the treatment of depression. First, some antidepressant drugs have been found to inhibit NMDA receptors (desipramine, mepyramine, milnacipran (Parsons et al., 1998)), second, chronic treatment with antidepressant produces alteration in NMDA receptors expression (Paul et al., 1994), third NMDA antagonists have antidepressive-like effects in animal models. These data find some conformation in a recent clinical trial showing short lasting psychotomimetic effects (hours) and long-lasting antidepressive action (days) following iv. infusion of ketamine as recently shown by Krystal's group (Berman et al., 2000). MRZ 2/579 shows clear, dose-dependent antidepressive-like activity in the Porsolt test but only the dose of 5 mg/kg was selective (devoid of locomotor stimulatory properties in the open field test) (Maj et al., not published). Similar effects were reported previously with memantine (Moryl et al., 1993).

The major concern for the clinical use of NMDA receptor antagonists is the side-effect profile. One of the major side-effects is psychotomimetic action which can be monitored in animals using prepulse inhibition of the acoustic startle reflex (PPI). (+)MK-801 and PCP produce clear impairment of PPI while MRZ 2/579 was not effective at the maximal tested dose of 20 mg/kg (Wedzony et al., not published). Similar results were obtained with memantine (ibid.).

Most of the studies presented above do not show clear advantage of low over high affinity NMDA receptor antagonists with exception of morphine self-administration which is inhibited by former agents only. However, the difference become evident when beneficial and side-effects are compared. It turns out that low affinity blockers have much better therapeutic index (therapeutic window) and at moderate doses are devoid of several side-effects

seen following high affinity antagonists administration. The following examples illustrate this claim:

1. comparison of neuroprotective and learning impairing potencies of (+)MK-801 and memantine leads to the conclusion that memantine has over 5 times wider therapeutic window (see Parsons et al., 1999).
2. At doses relevant for behavioural experiments in animal models PCP and (+)MK-801 but not memantine or MRZ 2/579 inhibit PPI.
3. In contrast to PCP and (+)MK-801 memantine in rats does not generalize to cocaine in drug discrimination studies (Koek et al., 1989; Sanger et al., 1992), and generalization to PCP occurs only at dose producing motor impairment (see Parsons et al., 1999).

Of course there is no proof that the differences outlined are directly related to the kinetics of the NMDA channel blockade, but it is an attractive and sound hypothesis.

Are low affinity better? In our opinion the answer is "*yes*". However, one should keep in mind the following:

1. lower affinity is only one of the determinants of good clinical tolerability. Other factors such as kinetics, voltage dependence, partial trapping, subtype selectivity and other factors definitely also play a role.
2. decreasing affinity leads unequivocally to a decrease in selectivity, namely action on other ion channels. This could be beneficial supporting therapeutic efficacy (good dirty drug) or could be detrimental if the "wrong" targets are hit. Hence, in our opinion there is certain optimal range of affinity at NMDA receptor which can be considered optimal – approx. from 0.5μM to 10μM.
3. The advantage of using NMDA channel blockers is their use dependent and voltage dependent mode of action which allows inhibition of pathological processes leaving physiological transmission preserved. However, it is unlikely that uncompetitive NMDA receptor antagonists with high subtype selectivity can be developed.
4. The fact that most of the registered agents affecting glutamatergic transmission are NMDA channel blockers is encouraging for new developments in this area. This is exemplified by the fact that there are currently over 10 agents of this type launched or at late stages of clinical development.

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Received August 31, 1999