Cognitive deficits are among the most debilitating and enduring impairments following human traumatic brain injury (TBI). In fact, disturbances in memory and cognitive function are the most frequently reported complaints of this population. Furthermore, these deficits may last for several years following the initial injury. In animal models of TBI, cognitive deficits have been produced following brain injury as shown by deficits in a number of learning and memory tasks.

Although much research has focused on investigating the acute biochemical changes that may induce cognitive dysfunction, efforts are just beginning to explore the physiological changes occurring at later intervals after trauma that may contribute to the maintenance of cognitive deficits. The evidence from this line of research suggests that specific deficiencies in central cholinergic neurotransmission may mediate cognitive dysfunction during the chronic period (days or weeks) after the initial insult. For instance, decreased hippocampal M1 binding has been observed 24 hours after injury in the rat. At 15 days following injury, Jiang, et al. observed an increase in Bmax of muscarinic acetylcholine receptors (mAChRs) in rat hippocampus, which may reflect a compensatory response to diminished cholinergic function. In addition, choline acetyltransferase immunoreactivity in the medial septal nucleus is decreased at 10 days following fluid-percussion TBI. Recently, a decrease in maximum velocity of choline uptake by cholinergic neurons in the rat hippocampus at 2 weeks after lateral cortical impact injury has been reported. These alterations in cholinergic function are particularly noteworthy given that prominent cognitive deficits are typically observed in animals at these time points.

Based on these recent observations of altered cholinergic function following TBI, administration of pharmacological compounds that act to improve aspects of cholinergic tone may prove beneficial for attenuating the perpetuation of traumatically induced cognitive deficits. For example, applied GABA inhibits central acetylcholine (ACh) release in animals. In addition, agonists that facilitate the activity of the GABA_A-benzodiazepine (BZD) receptor complex, such as muscimol or 4,5,6,7-tetrahydroisooxazol-5,4-c-pyridin-3-ol (THIP), have...
potent cholinergic-inhibiting properties and impair memory in animals.\textsuperscript{32,48} Therefore, diminishing this inhibitory control over cholinergic activity, or disinhibition, may facilitate cholinergic neurotransmission and in turn enhance cognition.\textsuperscript{41,42}

The aim of this experiment was to increase postinjury cholinergic function by using a negative modulator at the GABA\textsubscript{A} receptor. The compound MDL 26,479 (Suritoxol) is a negative modulator at the GABA\textsubscript{A} receptor that fulfills most of the requirements for a BZD receptor inverse agonist but lacks the proconvulsive or axiogenic properties characteristic of such compounds.\textsuperscript{31} This drug has been shown to increase cholinergic activity and enhance cognition in animals.\textsuperscript{19,31,34} The purpose of this experiment was to evaluate the therapeutic potential of postinjury treatment with MDL 26,479 for attenuating spatial memory deficits following TBI in the rat.

Materials and Methods

Experimental Animals

Sixty-four male Sprague Dawley rats weighing between 300 and 350 g were used. The animals were individually housed at 20˚ to 22˚C with a 6:00 to 18:00 light-dark cycle. Food and water were available ad libitum.

Apparatuses Used

Fluid-Percussion Device. The fluid-percussion device used to produce experimental brain injury was identical to that previously applied in rodents and is described in greater detail elsewhere.\textsuperscript{8} Briefly, the device consisted of a plexiglass cylinder reservoir 60 cm long and 4.5 cm in diameter. At one end of the cylinder was a rubber-covered plexiglass piston mounted on O-rings. The opposite end of the cylinder had a metal housing 2 cm long that contained a transducer. Fitted at the end of the metal housing was a 5-mm tube with a 2-mm inner diameter that terminated with a male Luer-Lok fitting. This fitting was connected to a female Luer-Lok fitting that had been chronically implanted over the exposed dura of the rat (see below). The entire system was filled with isotonic saline. The injury was produced by a metal pendulum that struck the piston of the injury device. The injury device injects a volume of saline into the closed cranial cavity and produces brief displacement and deformation of brain tissue. The magnitude of injury was controlled by varying the height from which the pendulum was released. The resulting pressure pulse was recorded extracranially by a pressure transducer (model EPN-0300A*, Grant Instruments, Cambridge, U.K.). Two stainless steel screws were placed 1 mm rostral to the bregma and 1 mm caudal to the lambda. A modified Luer-Lok syringe hub with a 2.6-mm inside diameter was placed over the exposed dura andbonded in place with cyanoacrylate adhesive. After the acrylic hardened, the injury tube was closed with Gelfoam, and the scalp sutured closed over the injury tube. Bacitracin was applied to the wound, and the animal was returned to its home cage.

Twenty-four hours after surgical preparation, the animals were anesthetized (4% isoflurane in a carrier gas mixture of 70% N\textsubscript{2}O and 30% O\textsubscript{2}). Rats in the sham-injury group were anesthetized and connected to the injury device, but the pendulum was not released. Those in the fluid-percussion injury group were anesthetized and injured at a moderate (2.1 atm) level of TBI. Previous studies have documented that this magnitude of injury produces acute hypotension, bradycardia, increased plasma glucose levels, motor deficits that last 5 to 7 days, and cognitive impairment lasting weeks.\textsuperscript{8,16} In addition, the behavioral deficits produced by this model occur in the absence of cell loss or axonal injury in the hippocampus.

Drug Treatment

For these experiments, MDL 26,479 was suspended in a 10-ml volume of distilled H\textsubscript{2}O and Tween 80 (1 to 2 drops of Tween per 5 ml distilled H\textsubscript{2}O). The suspensions were sonicated for 10 minutes.

Delayed Dosing. In the delayed dosing procedure, four groups of eight animals each were evaluated: sham-injured; injured, saline-treated; injured, 5 mg/kg MDL 26,479–treated; and injured, 10 mg/kg MDL 26,479–treated. These doses were selected based on previous reports that MDL 26,479 dose-dependently attenuated a delay-induced deficit on T-maze performance in rats.\textsuperscript{33} Drug-treated animals received the appointed dose of drug 60 minutes before each day’s testing in the Morris water maze (Days 11–15 postinjury) and saline-treated animals received an equal volume of 0.9% saline.

Early Dosing. In the early dosing procedure, four groups of eight rats each were also evaluated: sham-injured; injured, saline-treated; injured, 5 mg/kg MDL 26,479–treated; and injured, 10 mg/kg MDL 26,479–treated. All drug-treated animals were given the appointed dose of drug beginning 24 hours after injury and continuing through Day 15 postinjury. The drug was given 60 minutes before behavioral testing in the Morris water maze on Days 11 to 15 following injury. In addition, a group of sham-injured animals were treated with 10 mg/kg of MDL 26,479 and tested on Days 11 to 15 on the behavioral task.

Statistical Analysis

The swim latencies (in seconds) of the animals in locating the hidden platform for each group in the Morris water maze were analyzed by a 4 (Group) × 5 (Day) split-plot analysis of variance (ANOVA) for statistical comparisons. If a significant effect was found in the ANOVA, separate univariate ANOVAs were used for subsequent group comparisons. The Dunn–Sidak multiple comparison test was used to control for multiple univariate contrasts. A significance level of p < 0.05 was used for all tests.

Results

Figure 1 left presents the mean maze latency data (± standard error of the mean) for the delayed-dosing procedure. Analysis of the swim latency data yielded a signifi-
The significant main effect of group (F (3,27) = 8.577, p < 0.001). Subsequent group comparisons indicated that the data for the injured, saline-treated group were not significantly different from those of either of the two injured, MDL 26,479–treated (5 mg or 10 mg) groups (p = 0.1789 and p = 0.1685, respectively). Furthermore, the two drug-treated groups did not differ significantly in their performance (p = 0.1436). The significant overall group effect is a result of the sham-injured group having significantly shorter latencies to reach the goal platform compared to all other groups (p < 0.05 for all comparisons).

Figure 1 right presents the maze latency data for the early-dosing procedure. This analysis indicated a significant main effect for group (F (3,28) = 8.378, p < 0.001). Subsequent group comparisons indicated that both drug-treated groups (5 mg and 10 mg) had significantly shorter latencies to reach the goal platform than the injured, saline-treated group (p < 0.05 for all comparisons). The two drug-treated groups, however, did not differ in their goal latencies (p > 0.05). Both the injured, 5 mg- and injured, 10 mg-treated groups did have significantly longer latencies compared to the sham-injured group (p < 0.05 for both comparisons). Finally, the sham-injured, 10 mg-treated group and the sham-injured, saline-treated group did not differ in their latencies to reach the goal platform (p > 0.05).

Discussion
The present experiment examined the effect of delayed (Days 11–15) and early (Days 1–15) chronic postinjury administration of MDL 26,479, a negative modulator at the GABA<sub>A</sub> receptor, for attenuating spatial memory deficits following TBI in the rat. Results indicated that delayed treatment with either 5 or 10 mg/kg of MDL 26,479 beginning on Day 11 after injury was not effective in reducing cognitive deficits in injured animals. However, when either of these doses was given chronically beginning on Day 1 postinjury, it was effective in reducing cognitive impairment in injured animals.

It is unknown why delayed treatment with MDL 26,479 was ineffective in reducing cognitive deficits in injured animals. Perhaps in this injury paradigm, delaying drug administration until 11 days postinjury misses the therapeutic window for observing beneficial effects with negative modulators of the GABA<sub>A</sub> receptor. Delayed pharmacological manipulations have been successful in reducing neuronal damage following other types of brain injury, however. For example, a nootropic compound given several days following rodent lateral fluid-percussion injury improved Morris water maze performance.37 Other investigators have found that delayed administration with N-methyl-d-aspartate (NMDA) or amino-3-hydroxy-5-methyl-4-isozole propionic acid (AMPA) antagonists can attenuate neuronal damage following ischemia or lateral fluid-percussion injury, respectively.24,46 Ischemic injury is characterized by delayed neuronal death,39 therefore, there may be a longer window of opportunity to demonstrate effectiveness with delayed pharmacological interventions.

An alternative explanation is that MDL 26,479 may be.
effective when given at delayed postinjury intervals if a longer treatment regimen is employed. This is relevant to the methodological issues of the present experiment. With the early dosing protocol that was effective in attenuating cognitive impairment in brain-injured rats, the animals not only received the drug at an earlier time period but also received more injections over days (15 vs. 5 days) than those animals in the delayed dosing group. Thus, it is not known if drug treatment that is delayed yet given for a longer period of time would yield beneficial results. Additional experiments are required to investigate this possibility.

The beneficial effects observed with early chronic administration of MDL 26,479 are comparable to results obtained in other studies showing neurobehavioral protection with chronic drug administration. For example, chronic administration of the muscarinic cholinergic M₁ selective antagonist BIBN-99 to injured rats 1 to 15 days after injury has reduced deficits in Morris water maze performance.38 Furthermore, Liu, et al.25 found that chronic administration of cytosine diphosphate (CDP)-choline, a choline precursor, delivered to injured rats on Days 1 to 18 postinjury also improved spatial memory performance. Thus, use of a prolonged treatment strategy may be necessary to promote cognitive enhancement following TBI.

The novel triazole MDL 26,479 interacts with the GABA_A receptor in a similar manner to BZD inverse agonists. The physiological characterization of this agent has shown that MDL 26,479 inhibited in vivo binding of the BZD antagonist [³H]flumazenil to GABA_A receptors. This effect was observed when MDL 26,479 was given 6 hours before the radioligand, with maximum inhibition occurring 1 hour after drug administration. These authors have suggested that a possible metabolite of the parent compound, which produces these effects,31 may be active at GABA_A receptors. Although additional studies are needed to determine the precise mechanism of action for MDL 26,479 that is responsible for its cognitive-enhancing effects, the available data gathered thus far indicate that BZD inverse agonists may enhance cognition by selectively disinhibiting cholinergic neurons in the basal forebrain.40 Because MDL 26,479 is similar to BZD inverse agonists, this drug may produce its cognitive-enhancing effects by a similar mechanism. In addition, the compound's ability to increase [³H]hemicholinium-3 binding in the cortex may result from disinhibition of basal forebrain cholinergic neurons.31 This effect was blocked by the BZD antagonist flumazenil, suggesting that MDL 26,479's action is at GABA_A receptors. Tritiated hemicholinium-3 binds to the high-affinity choline uptake (HACU) site on cholinergic neurons; HACU is a regulatory and rate-limiting step in the synthesis of ACh, and its activity in vitro reflects activity levels of cholinergic nerve terminals in vivo.43 Increased turnover of ACh in these terminals leads to increased HACU and thus increased [³H]hemicholinium-3 binding. Several BZD inverse agonists that improve memory performance in animals have been shown to increase HACU; therefore, this may be a common mechanism of action for producing cognitive enhancement.26,30

A negative modulator at the GABA_A receptor, MDL 26,479 and its mechanism of action may involve the transsynaptic modulation of cholinergic afferents to various structures. The basal forebrain consists of a number of critical areas, any one of which could be the target for disinhibition by negative modulatory compounds such as MDL 26,479. In a previous study, Holley, et al.19 found that MDL 26,479 improved learning of a conditional visual discrimination task in rats with lesions of the nucleus basalis and substantia innominata. The nucleus basalis provides the major source of cholinergic innervation to the cerebral cortex. Thus, it may be speculated that previous reports of increased [³H]hemicholinium-3 binding in cortex by MDL 26,479 may be accomplished by disinhibiting cholinergic neurons in this brain region. In fact, disinhibition by MDL 26,479 may affect cholinergic neurons and their respective targets in a variety of basal forebrain structures.

Although it is enticing to attribute the beneficial effects of MDL 26,479 on cognitive function following TBI to enhancing cholinergic functioning, this speculation must be interpreted with caution. The impairments observed following TBI are not the result of deficits in a single neurotransmitter system.11,12,18 Accordingly, a drug manipulation at GABA_A receptors may affect a variety of neurotransmitters and/or processes. Although the physiological profile of the drug suggests that it may produce its beneficial effects by disinhibiting cholinergic neurons in the basal forebrain, the drug also augments in vitro long-term potentiation (LTP) in rat hippocampus.31 Long-term potentiation is considered an electrophysiological correlate of learning and memory.2 The drug’s cognitive-enhancing effects combined with its effects on LTP are intriguing given the enduring suppression of LTP that occurs following fluid-percussion TBI.33 Thus, it cannot be excluded that MDL 26,479 may produce cognitive enhancement by increasing LTP.

Improved neurobehavioral outcome with MDL 26,479 treatment supports a recently emerging hypothesis regarding pharmacological treatment of cognitive deficits following TBI. Given the literature implicating the cholinergic system in memory functioning6,10 and the suggestion that deficits in cholinergic functioning may contribute to neurological dysfunction following TBI,11,12,38 postinjury enhancement of cholinergic neurotransmission may provide one avenue for reducing cognitive dysfunction after head injury. This is in contrast to the well-documented beneficial effects of preinjury mAChR and the excitatory amino acid (EAA) receptor antagonist therapy approaches to treatment for behavioral deficits.16,26 Although ACh and NMDA receptor antagonists promote behavioral and cognitive recovery when administered just prior to or within minutes after injury, these same compounds are ineffective or even deleterious when administered at later intervals after injury. For example, scopolamine administered to brain-injured rats at 35 days postinjury disrupted Morris water maze performance.7 In addition, administration of the EAA antagonist MK-801 at 8 days postinjury impaired retention of a passive avoidance task in injured but not in uninjured rats.17 Such pharmacological probes suggest that excitatory receptor antagonist therapy may be an inappropriate strategy for reducing neuronal damage specifically when they are administered at delayed intervals after injury, although there are some exceptions.24 Moreover, the therapeutic window for observing beneficial effects with receptor antagonists may be longer in
humans than in animals. Nevertheless, the effects of MDL 26,479 in this experiment are particularly encouraging in that drug administration can be delayed for a period of time after injury and still produce beneficial results later when other treatment strategies are found either not feasible or perhaps even detrimental.

Whereas treatment with MDL 26,479 was beneficial in reducing cognitive deficits, performance of animals in the drug-treated groups did not reach levels of uninjured controls except for the last day of testing. The absence of continued improvement in the performance of injured animals given a higher drug dose suggests that decreasing GABAergic neurotransmission will not completely alleviate traumatically induced cognitive dysfunction. Thus, to increase the magnitude of cognitive recovery, a combination of drug treatments or pharmacological “cocktails” including compounds from several receptor systems may further enhance recovery. In fact, a combination of early receptor antagonist treatment and later treatment with compounds such as MDL 26,479 may prove beneficial if incorporated into a holistic treatment regimen for promoting cognitive recovery.

The clinical syndrome of cognitive deficits following head injury in humans is significant and persistent. The present study demonstrated that chronic postinjury treatment with MDL 26,479, a negative modulator at the GABA receptor, successfully attenuated cognitive deficits following rodent TBI. These results indicate that administration of MDL 26,479 is an effective postinjury intervention for reducing cognitive impairment following TBI and may have clinical utility as a postinjury treatment for the prolonged cognitive impairment present after head injury in humans.

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References

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34. Moran PM, Kane JM, Moser PC: Enhancement of working memory performance in the rat by MDL 26,479, a novel compound with activity at the GABA\textsubscript{A} receptor complex. Brain Res 569:156–158, 1992


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