Novel synaptic plasticity enhancer drug to augment functional recovery with rehabilitation

CURRENT OPINION

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Purpose of review
Stroke is a devastating illness which severely attenuates quality of life because of paralysis. Despite recent advances in therapies during acute phase such as thrombolytic therapy, clinical option to intervene the process of rehabilitation is limited. No pharmacological intervention that could enhance the effect of rehabilitation has not been established. Recent articles, which are summarized in the review article, reported novel small compound which accelerates training-dependent motor function recovery after brain damage.

Recent findings
A novel small compound, edonerpic maleate, binds to collapsin response mediator protein 2 (CRMP2) and enhance synaptic plasticity leading to the acceleration of rehabilitative training-dependent functional recovery after brain damage in rodent and nonhuman primate. The clinical trial to test this effect in human is now ongoing. Future preclinical and clinical studies will delineate the potentials of this compound.

Summary
A novel CRMP2-binding small compound, edonerpic maleate, accelerates motor function recovery after brain damage in rodent and nonhuman primate.

Keywords
α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor, edonerpic maleate, rehabilitation, stroke

INTRODUCTION

Brain damage can result from various neurological conditions such as stroke and degeneration leading to severe paralysis which aggravates quality of daily life in patients. In stroke, therapies during acute phase such as thrombolytic therapy have been known as an effective option. However, clinical intervention during rehabilitation phase is still limited. Furthermore, no effective pharmacological intervention with small compound has yet been established.

The functional recovery with rehabilitative training after brain damage relies on neural plasticity [1]. Synapses are structural unit to convey information from a neuron to other neurons [2,3]. Upon a stimulation of presynaptic neuron, a neurotransmitter is released from synaptic vesicles to the synaptic cleft and binds to its receptors at the postsynaptic site leading to the responses of postsynaptic neurons. Glutamate is a major excitatory neurotransmitter in the brain. Postsynaptic glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors and N-methyl-D-aspartate (NMDA) receptors are major ionotropic glutamate receptors [4]. The strength of the connection between the pre and postsynapse depends on the number of AMPA receptors in the postsynaptic membrane [5]. Tetanic synaptic stimulation leads to the sustained enhancement of synaptic responses, called long-term potentiation (LTP), which is a most characterized synaptic event during plasticity [6]. Synaptic incorporation and addition of AMPA receptors is a molecular basis of LTP [7]. This molecular event also occurs *in vivo* [8]. When animal learn or experience novel events, neural plasticity is induced and AMPA receptors are trafficked into synapses leading to the alteration of synaptic efficacy [5,9–18].

Despite large body of research accumulation of synaptic AMPA receptor delivery as a molecular mechanism of synaptic plasticity, this molecular event has not been intensively targeted to screen

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KEY POINTS

- Compounds for the recovery from stroke can be evaluated with experience-dependent synaptic AMPA receptor delivery.

- CRMP2 binding compound, edonerpic maleate, facilitates experience-dependent synaptic AMPA receptor delivery.

- Edonerpic maleate accelerates rehabilitative training-dependent motor function recovery from brain damage.

small compounds to enhance neural plasticity potential leading to the acceleration of functional recovery after brain damage. Recently, a novel small compound, edonerpic maleate, is reported to facilitate synaptic AMPA receptor incorporation and results in the augmentation of functional recovery after brain damage in rodent and nonhuman primate [19▪▪].

Classical targets of small compounds to enhance rehabilitation effect

The effect of small compounds on motor function recovery after stroke has been investigated in animal models and clinical trials. There are numerous amounts of studies focusing on adrenergic, dopaminergic, and serotonergic systems to modify neural plasticity and to play crucial roles in motor learning [20,21]. As amphetamine increases the extracellular concentration of catecholamines by blocking their reuptake, modulation of catecholamines was hypothesized to enhance motor function recovery. Although some studies exhibited evidence of facilitative effects of amphetamine on motor function recovery after brain damage with rodents and cats, augmentation of rehabilitation with amphetamine has been limited in nonhuman primates and is inconsistent in humans [22–25].

The effect of L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor of dopamine, on motor function recovery has also been examined. Based on an in-vivo microdialysis study in rat frontal cortex which showed increases in noradrenaline after systemic administration of L-DOPA [26], L-DOPA was proposed to increase noradrenaline in synapses and possess the efficacy on motor function recovery without systemic side-effects. However, improvement of motor function recovery with L-DOPA has not been reported in animal models. Despite the lack of biological evidence, L-DOPA was tested in human patients which exhibited inconsistent results [27,28].

Serotonin is also expected to be a potential target for pharmacological rehabilitation. It is widely recognized that selective serotonin reuptake inhibitors (SSRIs) work as antidepressants. Serotonin was reported to mediate synaptic trafficking of AMPA receptors during cortical reorganization after visual loss in rodents [14], suggesting the potential of serotonergic system as a plasticity modulator. In the fluoxetine (an SSRI) in motor recovery of patients with acute ischemic stroke (FLAME) trial, which was a double-blind, placebo-controlled trial, chronic administration of fluoxetine after stroke enhanced motor recovery [29]. However, in the fluoxetine or control under supervision trial, which was a pragmatic, multicenter, parallel-group, double-blind randomized-controlled trial, oral administration of 20 mg fluoxetine administration did not alter the modified Rankin Scale at 6 months after stroke the onset of stroke as compared with placebo [30▪▪].

New targets for pharmacological intervention

Some recent studies have expanded the concept of pharmacological intervention for functional recovery. A previous report indicates that the transcription factor cAMP (Adenosine 3′,5′-cyclic monophosphate)-response-element binding (CREB) protein expressed in the peri-injured region in stroke model plays a crucial role in maintaining the recovery process [31]. In this study, the induction of CREB expression was suggested to lead to the reorganization of the lost cortical sensory maps presumably by the activation of the unique transcriptome genes related to neural excitability and plasticity.

Interestingly, C–C chemokine receptor 5 (CCR5) which is a coreceptor of HIV virus, could also be a target for the functional recovery. A previous report demonstrated that knocking down of CCR5 augments motor function recovery in a stroke model animals and cognitive improvements in animal models with traumatic brain injury [32▪▪]. Knocking down of CCR5 could stabilize the dendritic spines and upregulate CREB and dual leucine zipper kinase signaling. This result is further supported by the human genetic analysis. Loss of function mutation, CCR5-Δ32, in stroke patients resulted in better outcome after stroke. This study leads to the hypothesis that the Food and Drug Administration-approved CCR5 antagonist, maraviroc, could be a potential agent to enhance functional recovery after stroke [32▪▪].

The recovery process with rehabilitation can depend on a functional reorganization of intact brain regions. A previous study demonstrated that synaptic AMPA receptor delivery underlies the functional cortical reorganization after visual deprivation [14]. In this study, somatosensory system is sharpened after
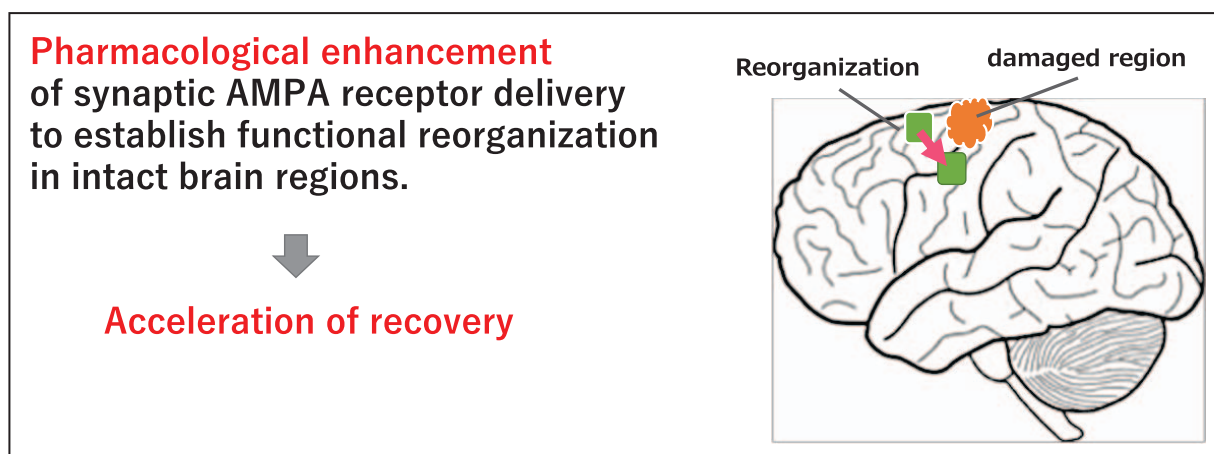
FIGURE 1. The novel concept of pharmacological intervention in stroke rehabilitation. The process of rehabilitation after brain damage includes a functional reorganization of intact brain regions. Once the neurons with functional connections are damaged, edonerpic maleate was able to recruit alternative neurons to compensate for the lost function by reorganizing the functional synapses in the intact brain region. This process was driven by accelerated training-dependent AMPA receptor delivery to the postsynaptic membrane.

visual deprivation which accompanies with synaptic AMPA receptor incorporation in the somatosensory cortex. This indicates that synaptic AMPA receptor delivery can mediate functional reorganization and compensation (Fig. 1). Further, experience-dependent synaptic AMPA receptor delivery occurs in other brain areas such as hippocampus and amygdala and can be a global mechanism of neural plasticity throughout the brain [9–11]. Thus, synaptic AMPA receptor delivery can be a novel target for the development of small compound to facilitate neural plasticity and ultimately to augment functional recovery after brain damage caused by various neurological conditions such as stroke.

Edonerpic maleate is a novel small compound to accelerate the functional recovery after brain damage

Recently, a small compound, edonerpic maleate, was reported to facilitate experience-dependent synaptic AMPA receptor delivery [19[**]]. The barrel cortex is a cortical area which receives and processes sensory input from whiskers in rodents. The barrel cortex consists of barrel-like columns. Each column primarily receives sensory input from a single whisker, and, thus, there exists a functional topographic mapping between each whisker and the corresponding barrel column [33–35]. This functional topographic mapping establishes in two weeks after birth, and during this process, whisker input drives AMPA receptors into synapses from layer 4 to 2/3 pyramidal neurons [8]. AMPA receptors form tetramers and have four subunits (GluA1–GluA4). GluA1 containing receptors (or GluA4 containing receptors early in

development) are selectively incorporated into these synapses with whisker experience [8,36]. This molecular event ceases after functional topographic mapping between a whisker and a barrel column is established. Therefore, natural whisker stimulation no longer drives AMPA receptors into synapses formed from layer 4 to 2/3 pyramidal neurons of the barrel cortex in adult animals [19[**]]. This indicates that a critical period exists in experience-dependent synaptic AMPA receptor delivery in the barrel cortex. The effect of edonerpic maleate on experience-dependent synaptic AMPA receptor delivery was examined using this feature. Under the administration of edonerpic maleate, natural whisker input appeared to drive AMPA receptors into layer 4 to 2/3 pyramidal synapses even in the adult barrel cortex of mice, thus, reopening the critical period of experience-dependent AMPA receptor delivery [19[**]]. This facilitative effect of edonerpic maleate on synaptic AMPA receptor delivery is tightly regulated by the sensory input, as synaptic AMPA receptor delivery did not occur in the absence of whiskers under the administration of edonerpic maleate [19[**]]. This unique characteristic of edonerpic maleate can be applied to accelerate functional recovery with rehabilitation after brain damage.

Cryoinjury was introduced to the motor cortex of mice which were trained with the reaching task using upper limb. Cryoinjury in the motor cortex attenuated the skill of reaching task with upper limb. Interestingly, the administration of edonerpic maleate promoted functional recovery after cryoinjury only in combination with a rehabilitative training [19[**]]. As reintroduction of cryoinjury in the brain region just rostral to the first injury area
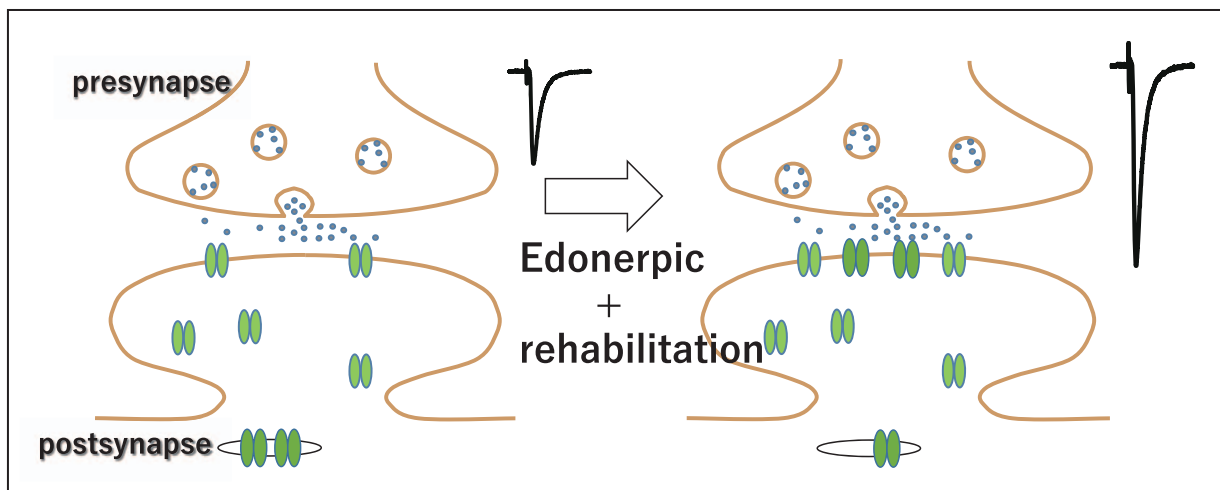
FIGURE 2. Administration of edonerpic maleate in combination with rehabilitative training drives AMPA receptors into synapses of circuits required for the functional recovery.

attenuated once recovered upper limb function in the reaching task, this region is supposed to be a compensatory brain area [19▪▪]. The amplitude of AMPA receptor-mediated synaptic currents was increased, and the expression of the cytoplasmic portion of GluA1, which blocks synaptic delivery of activity-dependent GluA1 containing AMPA receptors, prevented the functional recovery with edonerpic maleate [19▪▪]. These results demonstrated that edonerpic maleate drives training-dependent synaptic AMPA receptor delivery in the compensatory cortical areas leading to the functional recovery after cryoinjury (Fig. 2).

A molecular target of edonerpic maleate appeared to be collapsin response mediator protein 2 (CRMP2). CRMP2 was first identified as a downstream cytoplasmic molecule of semaphorin which has been well characterized as a repulsive molecule of developing axons and recently demonstrated to be involved in neural plasticity such as learning [37–39]. Edonerpic maleate binds to CRMP2 with moderate affinity [19▪▪]. Administration of edonerpic maleate failed to induce whisker experience-driven synaptic AMPA receptor delivery into layer 4 to layer 2/3 pyramidal synapses in the adult barrel cortex of CRMP2-deficient mice [19▪▪]. This indicates that the effect of edonerpic maleate on synaptic incorporation of AMPA receptor is mediated by the binding to CRMP2. In accordance with this, the administration of edonerpic maleate did not augment rehabilitative-dependent recovery of upper limb function of CRMP2-deficient mice with cryogenic injury of motor cortex [19▪▪]. This suggests that CRMP2 mediates acceleration of training-dependent motor function recovery after brain damage by edonerpic maleate.

How the complex of edonerpic maleate and CRMP2 facilitate experience-dependent synaptic AMPA receptor delivery? The activation of actin-depolymerizing factor (ADF)/cofilin increases actin turnover and mediates LTP-induced trafficking of AMPA receptors to the cell surface [40]. Considering that edonerpic maleate facilitates motor function recovery after brain damage in a training-dependent fashion, this compound could be active particularly under the plasticity inducing condition. Under chemical LTP condition, edonerpic maleate activated ADF/cofilin in the cortical slices. This activation is abolished in slices from CRMP2-deficient mice. Further, expression of dominant-negative form of ADF/cofilin prevented whisker experience-dependent synaptic AMPA receptors incorporation in the adult barrel cortex by edonerpic maleate [19▪▪]. The activation of ADF/cofilin was also detected in the peri-injured region of drug-administered mice with cryogenic injury which recovered in combination with rehabilitative training [19▪▪]. These results suggested that edonerpic maleate binds to CRMP2, activates ADF/cofilin, and facilitates input-dependent synaptic AMPA receptor delivery leading to the enhancement of motor function recovery after brain damage with rehabilitative training. It remains to be elucidated how the complex of edonerpic maleate and CRMP2 activates ADF/cofilin in a plasticity inducing condition. Influx and increase of intracellular calcium might trigger signaling pathways to activate ADF/cofilin by the complex of edonerpic maleate and CRMP2. Further, it remains to be elucidated if the effect of edonerpic maleate through CRMP2 is semaphorin-dependent or not. Semaphorin stimulation alters the phosphorylation status of CRMP2 [41]. Administration of edonerpic maleate in plasticity condition also alters phosphorylation status of CRMP2 [19▪▪]. Thus, edonerpic maleate might affect semaphorin signaling via CRMP2 phosphorylation.

These results suggest that CRMP2 can be a novel target to screen small compound which could enhance functional recovery in a training-dependent manner by enhancing synaptic AMPA receptor delivery driven by training. One interesting aspect of edonerpic maleate is that this compound could reopen the critical period of experience-dependent synaptic AMPA receptor delivery. The mechanisms underlying the determination and reopening of critical period is a biologically crucial question, and it will be interesting to elucidate how the complex of edonerpic maleate and CRMP2 reopens the critical period of experience-dependent synaptic AMPA receptor delivery.

A facilitative effect of edonerpic maleate on motor function recovery was also proven in the internal capsule hemorrhage model of nonhuman primate. Notably, dysfunction of precision grip with the index finger and the thumb of macaca monkey with internal capsule hemorrhage were prominently recovered by edonerpic maleate [19▪▪]. This movement with dexterity requires coordinated regulation of muscles with fine-tuning. Edenerpic maleate could strengthen specific neural pathways required for recovered movements with less nonspecific circuit activation, resulting in a decent signal to noise ratio among neural circuits. The effects of this compound on human patients need to be determined in ongoing clinical trials (JapicCTI-194633 and JapicCTI-194711).

CONCLUSION

Edonerpic maleate facilitates AMPA receptors incorporation into synapses in an experience and training-dependent fashion, leading to the acceleration of the recovery of voluntary movement after brain damage in combination with rehabilitative training. Clinical trials to evaluate the efficacy of edonerpic maleate in the recovery from stroke is being conducted. As the recovery process after spinal cord injury also involves functional cortical reorganization, edonerpic maleate might also be enhance the recovery from spinal cord injury and should at first be examined with animal models. To further screen next-generation compounds, the pharmacological and biological mechanisms of edonerpic maleate, including the signaling pathway to facilitate synaptic AMPA receptor delivery, should be investigated.

Conflicts of interest
There are no conflicts of interest.

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- ▪ of special interest
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