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LIGA20, a lyso derivative of ganglioside GM1, given orally after cortical thrombosis reduces infarct size and associated cognition deficit

(photothrombosis/sensorimotor cortex/water maze test/glycosphingolipids)

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ABSTRACT **A bilateral photochemically induced thrombotic lesion of rat sensorimotor cortex (≈3 mm in diameter and 25 mm³ in volume) is associated with a persistent cognition (learning and memory) deficit, which was evaluated with water maze tasks. The *N*-dichloroacetylsphingosine derivative of lysoGM1 (LIGA20) administered after the lesion either i.v. or *per os* reduces the infarct size by 30-40% and attenuates the associated cognition deficits, presumably by limiting the extent of damage of neurons at risk located in the surroundings of the infarcted core (i.e., area penumbra). The LIGA20 protection is dose and time dependent. Maximal protection is afforded by a single dose of LIGA20 of 34 μmol/kg i.v. 1 hr after lesion or by a dose of 270 μmol/kg *per os* when administered 1 hr and 24 hr after the lesion. The protective effect of LIGA20 can be observed when the drug is administered i.v. up to 6 hr after the lesion. The protective efficacy of the oral administration of LIGA20 is related to its physicochemical properties, which, unlike those of GM1, allow absorption from the gastrointestinal tract. LIGA20 given orally reaches the brain promptly and rapidly inserts into the neuronal membranes. Here, by an unknown molecular mechanism, LIGA20 selectively reduces the pathological amplification of Ca^{2+} signaling elicited by persistent stimulation of ionotropic glutamate receptors in the area penumbra.**

Natural (GM1, GD1a, GD1b, and GT1b) and semisynthetic (LIGA4 and LIGA20) glycosphingolipids protect neurons against excitotoxicity with high potency and efficacy when they are added to primary neuronal cultures prior to their exposure to excitatory transmitter amino acids including glutamate (1-4). A similar protection is elicited by parenteral GM1 (II³-*N*-acetylneuraminosylgangliotetraosylceramide) administration to rats, cats, and dogs when glutamate in brain interstitial fluids is enhanced by a hypoxia secondary to trauma or to various forms of impairment of cardiovascular function, including the experimental occlusion of the middle cerebral artery (MCA) (5–12). In addition, GM1 administered parenterally to patients a few hours after spinal cord injury or stroke can improve functional recovery (13–15). Since no treatment is available to limit brain or spinal cord excitotoxic damage, the protective action of GM1 and its derivatives deserves further study, particularly in view of their safety.

It is well established that the antiexcitotoxic action elicited by glycosphingolipids is due to a reduction of the pathological Ca^{2+} signal amplification step typical of excitotoxicity (16), and probably results from a combination of (*i*) a reduction of the pathological persistent activation and cell membrane translocation of protein kinase C, (*ii*) a facilitation of the impaired Ca^{2+} extrusion mechanisms including the sodium/calcium exchanger (10, 17), and (*iii*) a neurotrophic action related to a stimulation of membrane protein kinase autophosphorylation (18).

A unique property of glycosphingolipids is their ability to protect neurons from excitotoxicity without blocking glutamate channel-gating mechanisms or interacting with glutamate metabotropic receptors (1, 19). Thus, in distinction from competitive and noncompetitive glutamate receptor blockers, glycosphingolipids injected to experimental animals or to human subjects are virtually devoid of side effects.

Among the different GM1 derivatives tested *in vitro* as neuroprotective drugs the lyso derivative LIGA20 (II³-*N*-acetylneuraminosylgangliotetraosyl-D-*erythro*-1,3-dihydroxy-2-dichloroacetylamide-4-*trans*-octadacene), which includes the lipidic moiety *N*-dichloroacetylsphingosine, was found to be a more potent, faster acting, and longer lasting antiexcitotoxic agent than GM1 (2).

The characterization of LIGA20 physicochemical properties in water solution has revealed that the critical micellar size of this lysoGM1 derivative is more condensed than that of GM1 (20, 21). These properties reduce the probability of molecular self-aggregation and thus limit the molecular volume of these aggregates, thereby facilitating and accelerating their insertion in the lipid monolayer (20). Thus, the bioavailability of LIGA20 given orally to rats is at least 2 orders of magnitude greater than that of GM1 (22). Moreover, recent studies demonstrated that LIGA20 is slowly metabolized, and after a single oral administration a measurable brain content of this drug is maintained for longer than 48 hr (22). In addition, the parenteral or oral administration of LIGA20 to rats, before the induction of a photochemical brain infarct, exerts a strong neuroprotective action against glutamate-induced neurotoxicity in the areas that surround the region of maximal ischemic damage (10). In rats LIGA20 also reduces the size of brain lesions produced by MCA occlusion (23). In the present study, we investigated whether LIGA20 given *per os* or i.v. after the induction of a monolateral cortical photothrombotic infarct reduces the lesion size and in rats with bilateral cortical infarcts promotes the rehabilitation of the associated cognition deficit.

MATERIALS AND METHODS

Photochemical-Thrombotic Infarct. A focal brain lesion was produced as described by Watson *et al.* (24) with minor modifications [for details see Kharlamov *et al.* (10)]. A focused light beam (3 mm in diameter) was stereotaxically positioned on the exposed flat skull, 1.8 mm posterior to Bregma and 2.8 mm left of the midline. The underlying brain area corresponds to the left parietal sensorimotor neocortex. After the first 10 min of irradiation, the lamp was positioned on the right skull, 1.8 mm posterior to the Bregma and 2.8 mm right of the midline, and the light was turned on for an

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Abbreviation: MCA, middle cerebral artery.

additional 10 min. For the duration of surgery and irradiation, the animals were placed on a self-regulating temperature pad (CMA/150 temperature controller, Carnegie Medicine, Stockholm); the muscle temporalis temperature was stable at 37.4 ± 0.1°C. In a group of anesthetized rats that received rose bengal with or without LIGA20 but not irradiation, the brain temperature was determined 5, 10, 15, and 20 min after surgery. The brain temperature remained constant at 37.4 ± 0.1°C. Following light irradiation, animals were left to recover from anesthesia in a 37°C regulated chamber.

Morphometry. At different times after surgery, the brains were dissected into coronal 2-mm sections using a Jacobovitz brain slicer (Zivic-Miller). For the delineation of the infarct volume, the brain slices were incubated in buffered Locke's solution (pH 7.4) containing 2% triphenyltetrazolium chloride (Sigma) at 37°C for 15 min and then stored in 10% neutral buffered formalin (25). The size of the infarcted area for each slice was measured using a SAMBA-4000 computerized imaging system and SIGMA SCAN software with digitizing tablet (Jandel, Corte Madera, CA). The shape of the infarct zone in each slice is close to that of a frustum; therefore, its volume was obtained according to the following formula: $V = (\pi h/3)(r^2 + rR + R^2)$, where V = volume in mm^3, h = section thickness in mm, r = radius of the small base in mm, and R = radius of the large base in mm. In the slices containing the tip of the lesion, the infarct volume was calculated using the formula of the cone: $Bh/3$, where B is the area of the base of the cone in mm^2 and h is the height in mm. The total infarct volume was calculated by numerical summation of the volume calculated in each slice.

Brain Content of Glycosphingolipids. Extraction of brain glycosphingolipids was done at different times after oral administration of [^{3}H]LIGA20 or [^{3}H]GM1 (FIDIA S.p.A., Abano Terme, Italy) using the methods of Tettamanti et al. (26) as modified by Polo et al. (22). LIGA and GM1 were dissolved in saline and administered using an enteral feeding tube in a volume of 1 ml/150 g of body weight, in rats deprived of food for 6–8 hr.

Water Maze Test. A water maze test similar to that described by Kant et al. (27) was used. The maze consisted of opaque plastic walls arranged as three concentric squares set inside the water pool and equipped with removable doorways. The opening of the doorways allowed for the construction of mazes with an increasing number of potential error possibilities (see Fig. 3C). An "exit" platform was located at the end of the maze out of the level of the water line. During the training session (one trial a day for 7 consecutive days), unlesioned rats were placed in the center of the maze without alternative doorways open ("forced" maze) (Fig. 3C) and allowed a maximum of 3 min to reach the exit platform. Rats trained in this maze reach the exit platform in 15-20 sec and with only one to three incorrect choices. Testing with the forced maze enabled us to assess the animals' general motor, sensory, and motivational abilities and to exclude those animals unable to meet pretesting criteria. Performance is evaluated by the number of errors committed. Errors are defined as whole-body entry through doorways or into alleys not leading directly to the exit platform. On the 7th day after surgery, rats with bilateral sensorimotor cortical lesion were placed again in the same forced maze. Beginning from the 13th day after surgery, rats were subjected to a single trial every 4 days in a different maze constructed with alternative open doorways ("open" maze; Fig. 3C).

RESULTS

Effect of Glycosphingolipids on the Size of the Photothrombotic Lesion. The photothrombotic infarct obtained in the present experiments was parabolic in shape, had a very reproducible diameter of ≈3 mm (as indicated by the relatively small standard errors of the data listed in Table 1), and was consistently located in the sensory motor cortex. When LIGA20 is administered in an oral dose of 270 μmol/kg daily for 3 consecutive days and the last treatment is given 12 hr before the lesion, the size of the infarct measured 7 days after the lesion is reduced by 30-40% (Table 1).

In rats receiving repeated oral doses of LIGA20 of 270 μmol/kg, administered according to the dose regimen described in Table 1, the drug brain content is 650 ± 35 nM (n = 6). This value approaches the concentration required to protect primary neuronal cultures from glutamate-induced neurotoxicity (19), and it is significantly higher than the 250-300 nM concentration found in brain of rats receiving a single oral dose of LIGA20 of 270 μmol/kg 12 hr earlier (see Fig. 1).

The i.v. administration of LIGA20 at a dose of 34 μmol/kg 1 hr before the lesion also produces an infarct size reduction of 30-40% (Table 1). The extent of this protection is similar to that caused by dizocilpine, a noncompetitive N-methyl-D-aspartate receptor antagonist, given i.v. 1 hr before inducing the lesion (Table 1).

LIGA20 crosses the gastrointestinal wall and reaches the brain rapidly after oral administration; in contrast, oral GM1 is virtually not absorbed (Fig. 1). Therefore, to compare potency and efficacy of LIGA20 and GM1 given after the thrombotic lesion, we first administered these two drugs i.v. 1 hr after the lesion.

An i.v. dose of LIGA20 of 34 μmol/kg elicited a maximal protection (Table 1). Doses of 68 and 136 μmol/kg could not be more efficacious because 34 μmol/kg had already elicited the maximal possible protection. Intravenous administration of both LIGA20 and GM1 reduced the lesion size, but LIGA20 was significantly more potent than GM1; the minimal protective doses for LIGA20 and GM1 were 12 and 68 μmol/kg, respectively.

Most significant for its clinical implications, however, is the observation that in rats receiving LIGA20 (270 μmol/kg) orally 1 and 24 hr after the lesion, there is about a 30% reduction of the lesion size 7 days after the lesion (Table 1). The ability of LIGA20 given orally to reduce the cortical infarct size when given 1 hr after the lesion agrees with the observation that orally administered LIGA20 attains about a 200-300 nM brain concentration in a few hours (Fig. 1) and that LIGA20 injected i.v. (34 μmol/kg) after the photothrom-

Table 1. The photochemically induced cortical damage is reduced by i.v. or *per os* LIGA20 administered before or after the induction of the lesion

	Infarct size, mm^3			
	Saline	LIGA20		Dizocilpine
Treatment	*Per os* or i.v.	*Per os*	i.v.	i.v.
Before lesion*	27 ± 2.5	21 ± 2.1†	18 ± 4.8†	20 ± 5.8†
After lesion‡	25 ± 3.3	19 ± 0.9†	17 ± 3.7†	—

*Single i.v. injection of LIGA20 (34 μmol/kg) or dizocilpine (8.3 μmol/kg) was given 1 hr before surgery. Oral administration of LIGA20 (270 μmol/kg in 2 ml of saline) was carried out by stomach gavage once a day during 3 consecutive days; the last LIGA20 administration occurred 12 hr before inducing the infarct. Control groups received saline i.v. or by oral gavage in the same volume and with the same time schedule.

†$P < 0.05$ when compared with the respective saline-treated group (Duncan multiple range test).

‡A single i.v. injection of LIGA20 (34 μmol/kg) was given 1 hr after the lesion, and gavage of LIGA20 (270 μmol/kg) was carried out 1 and 24 hr after completion of the photothrombotic insult. Each value is the mean ± SE of at least 10 rats. Control rats received saline i.v. (n = 10) or *per os* (n = 10). Because the averages of the two groups were virtually identical, these values were combined. The size of the infarct was determined 7 days after the lesion.

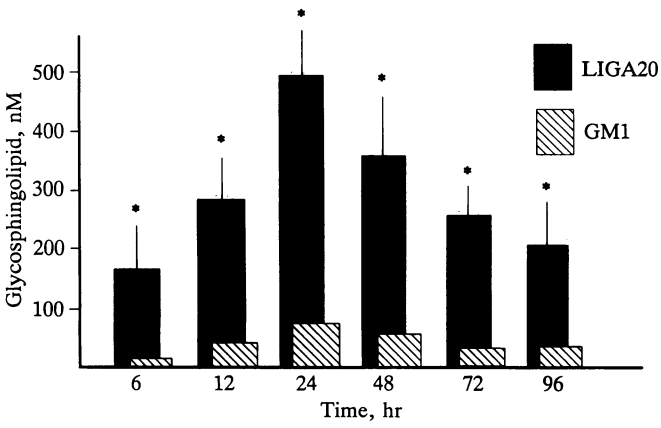

FIG. 1. Time course of brain glycosphingolipid content after oral administration of GM1 and LIGA20. LIGA20 (solid bars) or GM1 (hatched bars) (270 μmol/kg) was administered by oral gavage. Each value is the mean ± SE of three to five rats. *, $P < 0.01$ (the Student t test).

bosis has a time window of therapeutic efficacy lasting about 6 hr (Fig. 2).

Effect of Glycosphingolipids on the Cognition Deficit Associated with a Bilateral Cortical Photothrombotic Lesion. Bilateral sensorimotor cortex lesion of 25-30 mm^3 elicits a long-lasting cognition deficit that can be revealed by the rat performance in water maze tasks (Fig. 3).

To evaluate the effect of a LIGA20 treatment on the cognition deficit induced by the bilateral photothrombotic infarct, rats were trained for 7 days before the lesion (see *Materials and Methods*) to reach the exit platform in a water maze with closed doorways (forced maze; Fig. 3*C*). A trained rat reaches the exit platform in 15-20 sec and with only one to three incorrect choices (Fig. 3*A*).

On the 7th day after surgery, rats, placed again in the forced maze, retain the learned task (i.e., comparable number of errors committed before reaching the platform) whether they are sham-operated, lesioned without treatment, or lesioned and then treated with LIGA20 (Fig. 3*A*), indicating that the neurological deficit resulting from the lesion does not alter the performance. Beginning from the 13th day after surgery, the rats were subjected to one trial every 4-5 days in a maze with a different set of doorways opened (open maze; Fig. 3*A*).

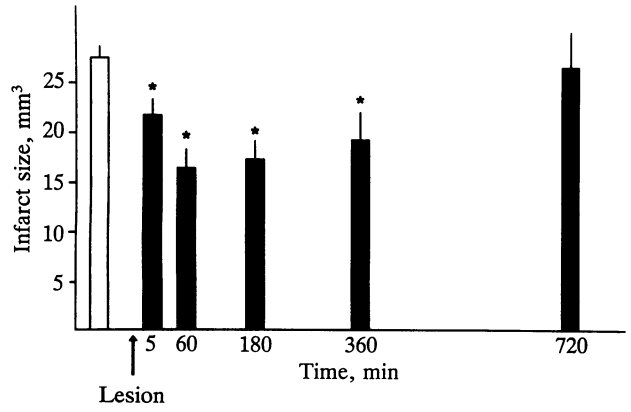

FIG. 2. Infarct size in rats treated i.v. with LIGA20 at different time intervals after the cortical photothrombotic lesion. The size of the infarct was determined 7 days after the lesion. Solid bars represent lesion volumes in rats receiving a single i.v. injection of LIGA20 (34 μmol/kg) at different time intervals after the lesion. Each value is the mean ± SE of at least 6 rats. The open bar represents lesion volume in saline-treated rats. The value for the saline-treated group is the mean ± SE of 12 rats, which were divided in three equal groups of animals receiving i.v. injection of saline at 5, 60, and 360 min after the lesion. *, $P < 0.05$ when compared with saline-treated group (Duncan multiple range test).

In the first trial with the open maze configuration, sham-operated rats reached the exit platform yielding a number of errors slightly greater than that recorded in the forced maze (Fig. 3*A*). However, rats with a bilateral sensorimotor cortex lesion manifested a highly significant performance impairment, indicating a retention deficit of the learned task (Fig. 3*A*). Importantly, both lesioned and nonlesioned rats appear to learn the novel maze task during the initial days of testing as demonstrated by a decrease in the number of errors. However, the rat performance up to 24 days after the lesion is consistently poorer than that of nonlesioned rats (Fig. 3*A*).

A single i.v. administration of LIGA20 at 34 μmol/kg 1 hr after the bilateral lesion decreases the size of the infarct (≈30%), which was measured 25 days after the lesion and elicits a comparable improvement in the water maze performance; both the number of errors and the learning time in the open maze configuration were reduced (Fig. 3*B*). Moreover, an i.v. injection of LIGA20 followed by repeated (once a day for 3 days) oral administrations of this glycosphingolipid, starting 1 hr after the bilateral lesion, is more potent than a single i.v. injection of LIGA20 in improving the cognition deficit and in reducing the lesion size (Fig. 3*A*). In an additional experiment (data not shown), we also observed that two oral administrations of LIGA20 at 270 μmol/kg 1 and 24 hr after a bilateral lesion decrease by ≈30% the size of the infarct and elicit an improvement in rat water maze performance that is comparable to the improvement observed after the i.v. administration of LIGA20 at 34 μmol/kg (Fig. 3*A*). In the experiment with oral LIGA20, rats were left without food for ≈6 hr before surgery; however, the ensuing hypoglycemia does not appear to play a role, because in vehicle-treated rats the 6- to 8-hr fasting, before the lesion, failed to change the size of the lesion and the water maze performance.

DISCUSSION

After a small photothrombotic lesion of the sensorimotor cortex, rats develop a cortical brain infarct in which three contiguous and almost concentric brain areas can be distinguished by the severity of the neuronal damage. The first area is characterized by a primary microthrombotic ischemic core of 1-1.5 mm in radius where the majority of cells are dead (see also ref. 10). This area, which has sharp boundaries, mimics the microthrombotic focal infarct observed in some forms of human stroke (28). Importantly, in our model, between 60% and 70% of the final infarcted volume measured 7 days after the lesion is attributable to the initial irreversible neuronal degeneration that occurs almost instantaneously after the photothrombotic insult. This primary lesion not only develops rapidly and is irreversible but also is resistant to any pharmacological treatment initiated after the lesion. The second area surrounds the primary infarct (i.e., area penumbra) and is about 1 mm in radius (10). In this region the cortical architecture is relatively preserved at first, but extensive neuronal death is impending and materializes within 18-48 hr (10, 28). Unlike the ischemic core, the progression of the neuronal damage within the area penumbra is dependent, inter alia, from the elevated glutamate concentrations in the interstitial fluids and is susceptible to pharmacological treatments; thus, it is this region that is the target of the glycosphingolipid treatment. In the absence of a pharmacological intervention, the area penumbra is incorporated in the infarcted area and in our model may contribute up to 30-40% of the final infarct volume. This feature of the photochemical lesion might explain why the maximal reduction of the infarct size afforded by a treatment with glycosphingolipids or dizocilpine never exceeds a 30-40% reduction of the infarct

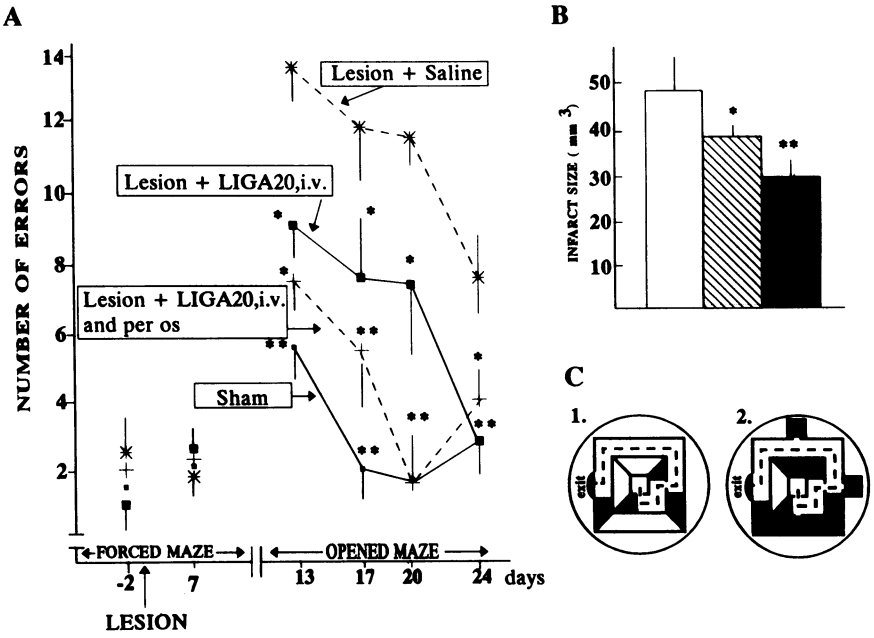

FIG. 3. Treatment with LIGA20 after the bilateral photothrombotic cortical lesion reduces the infarct size and improves the cognition deficit associated with a bilateral sensorimotor cortex lesion. (*A*) Rats were trained for 7 days, once a day, in a forced maze (see *Materials and Methods* for details) before lesioning. Animals were scored for performance 2 days before the lesion (−2 on *y* axis). Rats were then divided randomly in four groups: (*i*) Sham, received rose bengal plus saline or LIGA20 but not skull exposure to the white light. (*ii*) Lesion + Saline, received bilateral photothrombotic lesion and saline i.v. or *per os* starting 1 hr after termination of the lesion. (*iii*) Lesion + LIGA20, i.v., received LIGA20 (34 μmol/kg) 1 hr after termination of the bilateral photothrombotic lesion. (*iv*) Lesion + LIGA20, i.v. and per os, received LIGA20 i.v. (34 μmol/kg) 1 hr after the lesion and LIGA20 *per os* (270 μmol/kg) 24, 48, and 72 hr later. Seven days after the lesion, rats were exposed again to the water forced maze test; at days 13, 17, 20, and 24 after the lesion, they were exposed to an open maze test. (*B*) The infarct volumes (right and left sensorimotor cortices) in the different groups were determined 25 days after the induction of the photothrombotic lesion. The open bar represents the volume of the bilateral lesion in rats receiving saline (group *ii* in *A*). The hatched bar represents the volume of the bilateral lesion in rats receiving single i.v. injection of LIGA20 (group *iii* in *A*). The solid bar represents the volume of the bilateral lesion in rats receiving combined (i.v. and *per os*) treatment with LIGA20 (group *iv* in *A*). For each group the values are the mean ± SE of at least six rats. Statistical comparison among groups was carried out with the Duncan multiple range test. *, $P < 0.05$ when compared with Lesion + Saline group. **, $P < 0.01$ when compared with Lesion + Saline group. (*C*) Diagram of water maze configurations. 1., Forced maze; 2., open maze. The broken lines track the path the animals have to swim to reach the exit platform. Black regions denote error possibilities.

size. Contiguous with the area penumbra is a large reactive zone that does not appear sharply delineated anatomically and may extend to many areas of the cortical hemisphere ipsilateral to the lesion. The pathology of this area is characterized by minor morphological signs of damage, such as edema, which is resolved in 2–3 days (29), and alterations of various biochemical markers (10).

The cortical infarction produced in this model has several interesting features that make it appealing for the study of neuroprotective drugs. For instance, it provides a lesion of reproducible size in which the hemodynamic consequences (changes in cerebral blood flow, blood brain barrier permeability to proteins, and alteration of regional water and electrolytes content), the glucose utilization, and the area penumbra histopathological signs are similar to those described in the human stroke and in the rat model of MCA occlusion (6, 10, 11, 28–32). A vasogenic edema, which occurs 4 hr after the photochemical lesion, is evident in brain areas remote from the lesion and, as is the case in the MCA model, it may well induce a secondary ischemia in remote areas ipsilateral to the lesion. Although the cerebral infarction produced by the photochemical model is initiated by thrombosis of small blood vessels—and therefore in this respect it differs from the human stroke and from the MCA model—it, nevertheless, includes several of the main features of human stroke and MCA model and therefore can be used as a guide to evaluate dosage and treatment regimens to be tested in the human stroke. In the photochemical model adopted in this study, the capability to elicit a discrete and highly reproducible infarct size, in preselected cortical regions, bilaterally, facilitates the study of the drug action on the behavioral and functional consequences of a brain lesion.

The role of excitotoxins (i.e., glutamate) in enlarging the infarct size via a secondary neurodegeneration is demonstrated by the reduction of the cortical infarct volume in animals pretreated with dizocilpine (Table 1). In a previous study, using dizocilpine we have shown that after a photochemically induced thrombosis an increased and abnormal *N*-methyl-D-aspartate receptor stimulation persists in the area surrounding the focal lesion longer than 6 hr (10). Thus LIGA20 administered after the lesion reduces the glutamate-induced secondary neurodegeneration in the area penumbra if the drug is given within a window of therapeutic opportunity (Fig. 2), which appears to last up to 6 hr after the lesion.

The efficacy of orally administered LIGA20 in preventing the secondary neuronal degeneration in the area penumbra of the cortical photothrombotic infarct may be due to this drug's physicochemical properties, which allow establishment of high brain and blood drug concentrations in 6-12 hr after the injection (Fig. 1). In brain the drug concentration 12 hr after an oral administration of LIGA20 at 270 μmol/kg is ≈280 nM. Since the LIGA20 plasma levels are 4- to 6-fold greater than that of brain (22,23), one can presume that the drug concentration in the brain may not be uniform and perhaps may be higher in the surroundings of the infarcted area due to local changes in the blood-brain barrier. A protracted treatment combining i.v. (1 hr after the lesion) and oral (repeated 12, 24, and 48 hr after the lesion) administrations of LIGA20 not only reduced the lesion volume but also promoted a greater functional recovery than that observed after either a single i.v. injection of LIGA20 at 34 μmol/kg (Fig. 3 *A* and *B*) or two

oral administrations of LIGA20 at 270 μmol/kg 1 and 24 hr after the bilateral lesion (data not shown). Very likely the time window to reduce neuronal degeneration and promote rehabilitation of the associated cognition deficit resulting from the photochemically induced bilateral cortical infarct may be lasting longer than predicted from morphological studies of the time course of the infarct evolution. Indeed, the cortical area adjacent to the area penumbra includes neurons at risk of impending glutamate excitotoxicity as documented by the appearance of various dizocilpine-sensitive biochemical markers (10). The neurons that undergo a delayed slow degeneration may not contribute substantially to the total size of the lesion, but their integrity may be of paramount importance in the functional compensation to the primary damage and may be operative in the drug-induced cognition rehabilitation.

Alternatively LIGA20, similarly to GM1 (18), may act also as a neurotrophic factor by directly activating Trk receptor autophosphorylation, thereby mimicking the action of the nerve growth factor and other neurotrophins (18). By this mechanism LIGA20 may help to limit neuronal damage in the area penumbra and enhance cell survival and/or facilitate repair responses. The time window for such additional neurotrophic actions of LIGA20 might last several days after the excitotoxic insult (33–35).

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1. Manev, H., Costa, E., Wroblewski, J. T. & Guidotti, A. (1990) *FASEB J.* **4**, 2789–2797.
2. Manev, H., Favaron, M., Vicini, S., Guidotti, A. & Costa, E. (1990) *J. Pharmacol. Exp. Ther.* **252**, 419–427.
3. Scaper, S. D., Leon, A. & Facci, L. (1991) *J. Pharmacol. Exp. Ther.* **259**, 452–457.
4. Choi, D. W. (1988) *Neuron* **1**, 623–634.
5. Urbanics, R., Greenberg, J. H., Toffano, G. & Reivich, M. (1989) *Stroke* **20**, 795–802.
6. Karpiak, S. E., Mahadic, S. P. & Wakade, C. G. (1990) *CRC Crit. Rev. Neurobiol.* **5**, 221–237.
7. Bharucha, V. A., Wakade, C. G., Mahadic, S. P. & Karpiak, S. E. (1991) *Exp. Neurol.* **114**, 136–139.
8. Seren, M. S., Rubini, R., Lazzaro, A., Zanoni, R., Fiori, M. G. & Leon, A. (1990) *Stroke* **21**, 1607–1612.
9. Costa, E., Kharlamov, A., Guidotti, A., Hayes, R. & Armstrong, D. M. (1992) *Pathophysiol. Exp. Ther.* **4**, 17–23.
10. Kharlamov, A., Guidotti, A., Costa, E., Hayes, R. & Armstrong, D. M. (1993) *J. Neurosci.* **13**, 2483–2494.
11. Simon, R. P., Chen, J. & Graham, S. H. (1993) *J. Pharmcol. Exp. Ther.* **265**, 24–29.
12. Redmond, J. M., Gillinov, A. M., Blue, M. E., Zehr, K. J., Tronicoso, J. C., Camerond, E., Johnston, M. V. & Baumgartner, W. A. (1993) *Surgery* **114**, 324–333.
13. Geisler, F. H., Dorsey, F. C. & Coleman, W. P. (1991) *N. Engl. J. Med.* **324**, 1829–1838.
14. Carolei, A., Fieschi, C., Bruno, R. & Toffano, G. (1991) *Cerebrovasc. Brain Metab. Rev.* **3**, 134–157.
15. Alter, M. & The SASS Investigators (1994) *Stroke*, in press.
16. DeErausquin, G. A., Manev, H., Guidotti, A., Costa, E. & Brooker, G. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8017–8021.
17. Kiedrowski, L., Brooker, G., Costa, E. & Wroblewski, J. T. (1994) *Neuron*, in press.
18. Ferrari, G., Batistatou, A. & Green, L. A. (1993) *J. Neurosci.* **13**, 1879–1887.
19. Favaron, M., Manev, H., Alho, H., Bertolino, M., Ferret, B., Guidotti, A. & Costa, E. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 7351–7355.
20. Perillo, M. A., Polo, A., Guidotti, A., Costa, E. & Maggio, B. (1993) *Chem. Phys. Lipids* **65**, 225–238.
21. Sonnino, S., Cantu, L., Corti, M., Acquotti, D., Kirschner, G. & Tettamanti, G. (1990) *Chem. Phys. Lipids* **56**, 49–57.
22. Polo, A., Kirschner, G., Guidotti, A. & Costa, E. (1994) *Mol. Chem. Neuropathol.* **21**, 41–53.
23. Seren, M. S., Lazzaro, A., Yang, C. L., Canella, R., Bassan, M., Zanoni, R. & Manev, H. (1994) *J. Pharmacol. Exp. Ther.* **268**, 460–465.
24. Watson, B. D., Dietrich, W. D., Busto, R., Wachtel, M. S. & Ginsberg, M. D. (1985) *Ann. Neurol.* **17**, 497–504.
25. Bederson, J. B., Pitts, L. H., Germano, S. M., Nishimura, M. C., Davis, R. L. & Bartkowski, H. M. (1986) *Stroke* **17**, 1304–1308.
26. Tettamanti, G., Bonali, F., Marchesini, S. & Zambotti, V. (1973) *Biochim. Biophys. Acta* **296**, 160–170.
27. Kant, G. L., Yen, M. H., D'Angelo, P. C., Brown, A. J. & Eggleston, T. (1988) *Pharmacol. Biochem. Behav.* **31**, 487–491.
28. DeRyck, M. (1992) *Eur. Neurol.* **30**, Suppl. 12, 21–27.
29. Pierpaoli, C., Righini, A. & Linfante, I. (1993) *Radiology* **189**, 439–448.
30. Ginsberg, M. D. & Busto, R. (1989) *Stroke* **20**, 1627–1642.
31. Dietrich, W., Ginsberg, M. D., Busto, R. & Watson, B. D. (1986) *J. Cereb. Blood Flow Metab.* **2**, 184–194.
32. Dietrich, W. D., Watson, B. D., Busto, R., Ginsberg, M. D. & Bethea, J. R. (1987) *Acta Neuropathol.* **72**, 315–325.
33. Cecarelli, B., Aporti, F. & Finesso, M. (1976) *Adv. Exp. Med. Biol.* **71**, 275–293.
34. Scaper, S. D., Leon, A. & Toffano, G. (1989) *Mol. Neurobiol.* **3**, 173–199.
35. Garofalo, L. & Cuello, A. C. (1994) *Exp. Neurol.* **125**, 195–217.