Effects of Ginkgo biloba extract on cortical hemiplegia in the rat

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Summary — Interested in the pharmacological treatment of subjects with brain lesions, we studied the effects of chronic (7 and 30 d) treatments with Ginkgo biloba extract (EGb 761, Ipsen) in two animal models of cortical hemiplegia: one induced by motor-cortex aspiration and another using a reversible inactivation of the motor cortex through chronic, localized infusion of an inhibitory neurotransmitter, γ-aminobutyric acid (GABA), by means of osmotic minipumps.

The elevated beam test, which evaluates coordinated walking, was used in water-deprived animals trained to drink saccharin-sweetened solutions (with or without EGb 761) and to perform to criteria before the surgical procedures. Surgery involved either sham operation, unilateral motor-cortex aspiration or unilateral, chronic infusion of GABA into the same region. From the day after surgery, the rats were administered 100 mg/kg/d of EGb 761 daily for 7 or 30 d. The extract was spontaneously taken by the rats twice daily.

In all groups in which the extract was administered, a faster and more complete recovery from the motor deficits was observed, a result which was significantly different from that in rats to whom only saccharin solutions were given. No differences were detected for sensory deficits.

The beneficial effects of EGb 761 were more apparent in the motor-cortex aspiration groups than in the GABA-treated rats. Histological analysis showed that EGb 761-treated rats had significantly smaller ventricular diameters than untreated animals, although immunocytochemical analysis of gliotic reaction (GFAP staining) did not show group differences.

We conclude that EGb 761 has beneficial effects on recovery from cortical hemiplegia in the rat. However, EGb 761 active principle(s) and mechanism(s) of action remain to be further elucidated.

hemiplegia / motor cortex / functional recovery

Introduction

Pharmacological treatment of subjects with brain lesions has been the focus of intensive research in the last few years (for review, see Brailowsky, 1980; Feeney and Sutton, 1987).

Ginkgo biloba extract (EGb 761, Ipsen), prepared from the dry leaves of the tree Ginkgo biloba, has been shown to protect against brain damage caused by hypoxia...
(Clostre, 1986; Oberpichler et al, 1988) and to facilitate recovery from bilateral frontal lobotomy in rats (Attella et al, 1989).

In this chapter, we briefly describe our results with chronic (30 d) and semi-chronic (7 d) oral administration of a *Ginkgo biloba* extract (EGb 761) to rats in which a unilateral motor deficit was induced through two different somatomotor cortex interventions: the ‘classic’ one involving tissue aspiration, and a novel pharmacological technique of reversible functional inactivation using a continuous, chronic and localized infusion of γ-aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the brain, into the motor cortex of rats. With this approach we produced a syndrome of hemiplegia that lasts longer than the one produced by motor-cortex ablations (Brailowsky et al, 1986). Moreover, the interruption of this chronic GABA infusion gives rise to a compensatory excitability rebound, now known as the ‘GABA-withdrawal syndrome (GWS)’, in which focal epileptogenesis, induced by the abrupt interruption of cortical GABA infusions, has been observed both in rats and monkeys (Brailowsky et al, 1988).

**Materials and Methods**

**Subjects**
The experiments were performed in adult male Wistar rats, weighing 200–250 g at the time of surgery, obtained from the UNAM breeding unit. The rats were housed in polycarbonate cages, 2/cage, and kept at constant temperature conditions under a 12/12 h light–dark cycle. They were water-deprived but had free access to food.

Each of the 14 study groups described below consisted of 12 rats at the time of surgery: 1) sham group without EGb 761 (saccharin only), 14-d survival after surgery; 2) sham group without EGb 761 (saccharin only), 37-d survival after surgery; 3) sham group with EGb 761 for 7 d; 4) sham group with EGb 761 for 30 d; 5) motor-cortex (MCx) aspiration without EGb 761 (saccharin only), 14-d survival after surgery; 6) MCx aspiration without EGb 761 (saccharin only), 37-d survival after surgery; 7) MCx aspiration with EGb 761 for 7 d; 8) MCx aspiration with EGb 761 for 30 d; 9) unilateral GABA infusion for 7 d into the MCx without EGb 761 (saccharin only), 14-d survival after surgery; 10) unilateral GABA infusion for 7 d into the MCx without EGb 761 (saccharin only), 37-d survival after surgery; 11) same as group 9 with EGb 761 for 7 d; 12) same as group 9 with EGb 761 for 30 d; 13) EGb for 7 d, and induction of a GWS (see below) on the 7th d; 14) induction of a GWS followed by acute ip administration of a 100 mg/kg dose of EGb 761.

**Drug administration**
EGb 761, prepared as concentrated powder, was supplied by Ipsen (Paris). The powder was dissolved in water and saccharin (0.1%) and given orally at a 50 mg/kg dose twice a day in a total volume of 3 ml. The interval between the doses was 9–10 h. Sham rats received the same volume of saccharin solutions but without EGb 761.

**Testing and surgical procedures**

*Phase I.* The rats were given 7 d to become accustomed to the experimental room and the testing apparatus, and were exposed to the saccharin solution (3 ml) twice a day.

*Phase II.* The rats were trained for 7 d to walk on an elevated narrow (2.5 cm) wooden beam (2 m long), following the same procedure as that described previously (Brailowsky et al, 1986).
Performance was measured in terms of motor score (0 = no deficit to 6 = total incapacity) evaluated for each quarter of the beam (maximal score of 24), and time to reach the opposite end of the beam. The interobserver variability was taken into account, using group video sessions to standardize scoring criteria.

Phase III. At the end of phase II, when the animals had stable motor scores, the surgical procedures were performed. Once anesthetized (halothane), the rats were positioned in a stereotaxic apparatus using the flat skull position.

Motor-cortex aspiration. To standardize lesion size, we used a 22 G wire bent to form a square, 3 mm wide, mounted on a stereotaxic holder. Once the cortical tissue of the right side somatomotor region was exposed, the device was lowered 2 mm into the cortex; then the holder was moved anteriorly–posteriorly for 4 mm, to excise a cortical slab of 3 x 4 mm.

GABA infusion. Osmotic minipumps (Alza model 2001, delivery rate = 1 μl/h for 7 d), filled with ultrafiltered GABA (100 μg/μl) dissolved in saline (pH 7.33) and adapted with a catheter (PE60) at the outlet port, were incubated at 37°C in saline for at least 5 h before implantation. Under halothane anesthesia, a small trephine over the right side somatomotor region (2 mm posterior, 2 mm lateral from the bregma) was made. With a sterile needle, the dura was opened. The minipump was then implanted subcutaneously in the interscapular space. Its catheter was run under the skin to reach the skull, where it was inserted tangentially through the trepnie into the cortex for 2 mm from the bone surface.

Phase IV. This phase started on the day after surgery, with the oral administration of the saccharin solutions, either containing EGB 761 or not, for 7 or 30 d, according to the group. The rats were tested daily and were followed until the 7th day after the end of the treatment.

Electrode implantation
The rats in groups 13 and 14 were prepared for chronic EEG recording and intracortical infusion as reported previously (Brailowsky et al, 1988). Epidural screws were fixed to the skull and stainless steel cannulae (0.5 mm external diameter) were positioned in the hindlimb representation area of the somatomotor cortex (2 mm posterior to the bregma, 2 mm lateral, 1.5 mm deep from the bone surface). Group 13 rats were also habituated to drinking a saccharin solution containing the Ginkgo powder.

GWS induction
The GWS was induced by infusing GABA for 24 h, through minipumps as described above, and by interrupting this administration by catheter disconnection. The EEG was monitored (Grass model 78 polygraph) continuously until the appearance of focal epileptic activity. In the EGB 761-treated rats, EEG recordings were obtained every other day before and after intracortical GABA infusion. In group 14, EGb was administered ip 60 min after the beginning of the GWS.

Histological procedures
Three days after completion of phase IV, the rats were sacrificed with a barbiturate overdose and then perfused intracardially with cold, buffered saline followed by phosphate–lysine–paraformaldehyde (PLP) fixative for immunohistochemistry (glial fibrillary acidic protein; GFAP), according to the method of Hsu et al (1981) and for Nissl staining.

Lesion size, ventricular diameter and gliosis were determined using an MCID image analyzer (Imaging Research, Inc, Ontario, Canada).

Statistics
Data were analyzed using a non-parametric analysis of variance for related samples (Friedman test). When significant differences between groups were found (ie, P < 0.05), a Mann–Whitney U-test was applied for comparing treated vs untreated subjects (SPSS/PC package).
Results

Behavioral

Sham groups
EGb 761 was readily taken by the rats. Neither the 7-d nor the 30-d treatment had any apparent toxic behavioral effects and no significant differences in sensorimotor function were detected among these groups.

The first group we operated on was group 4 (sham + EGb 761 for 30 d); surgery was performed under barbiturate anesthesia (35 mg/kg, ip). These rats had transient (2–3 d) motor deficits and slower speed on the beam which were not observed in the animals we had operated on before under halothane anesthesia (data not shown). Therefore, halothane was used on the remaining animals.

Motor-cortex aspiration
From the short-survival group, all rats except the shams showed severe motor impairments contralateral to the operated side from the day after surgery. The group that showed the bigger and longer-lasting deficit (ie, higher scores) was that without EGb 761 treatment. The treated group had significantly better performances than the untreated group.

In the animals with longer follow-up periods and treatments (fig 1), the effect of EGb 761 was striking. The rats receiving EGb 761 were undistinguishable in their motor performance from the sham group after the 7th day of treatment except for 3 d, when an ‘escape’ from the drug effect was seen. The beneficial effect lasted until 7 d after the end of EGb 761 administration. Their speed was also significantly faster than that of untreated rats and comparable to that of the sham group from the 8th d after surgery.

Hemiplegia by GABA
In the rats which received EGb 761 for 7 d, no statistical differences among the hemiplegic groups were observed during the first 3 d of GABA infusion and the slopes of the recovery curves were similar. By d 4 after surgery, the group receiving EGb 761 (group 11) began showing better functional recovery than the other. This amelioration pattern persisted for 12 d after the beginning of GABA infusion.

For the motor-cortex aspirated rats with long follow-up (fig 2), no group differences in motor scores were detected until the 20th d after surgery. From that time on, the EGb 761-treated rats performed better than the untreated animals and comparably to the sham group.

Electrical

GWS groups
Chronic administration of EGb 761 did not protect the rats from showing a GWS upon GABA-infusion interruption (group 13) and, in the those in which a GWS was induced (group 14), EGb 761 (100 mg/kg, ip) given 60 min after the beginning of
**EGb 761 effects on cortical hemiplegia**

**MOTOR CORTEX ASPIRATION**

Fig 1. Motor performance in the elevated beam test by rats either sham-operated without any treatment (●) or with unilateral motor-cortex aspiration with (▲) or without (△) EGb 761 treatment for 30 d. Animals were trained before surgery and EGb 761 treatment started on the day after (d 9), lasting until d 39. Each point represents the mean ± SEM. Drug treatment facilitated functional recovery in EGb 761-treated groups. See the text for further details.

the epileptic discharges did not modify either the discharge frequency or the EEG pattern.

**Histological**

All lesions, either aspiration- or cannulae-induced, involved all cortical layers, and in a few cases, an extension into the corpus callosum. Lesion size in the former group was comparable and always comprised the somatomotor region. The minipump-infused groups also showed similar cortical pathology. No significant intragroup differences were seen for lesion size in the motor-cortex aspiration or in the GABA-infusion groups.

Ventrices of the motor cortex-aspirated rats showed clear asymmetries in the untreated rats, with the ventricle ipsilateral to the lesion being significantly larger. No such asymmetries were noted in the EGb 761-treated animals. However, when com-
Fig 2. Motor performance in the elevated beam test by rats with unilateral chronic GABA infusion into the motor cortex lasting for 7 d, with (▲) or without (△) EGb 761 treatment for 30 d (GABA + EGb 30), and control animals (●). Details are provided in the text.

paring the operated sides of both groups, a significant difference was obtained, with the treated group showing smaller ventricles. No such differences were noted in the GABA-infused rats.

When analyzed for chromatolysis and GFAP immunoreactivity, gliotic changes were observed around the cortical lesion but also in the thalamic projection areas (ventrolateral, ventrodorsal posterior and intralaminar groups). These distant lesions were more diffuse and showed more intense astrocyte reaction in the short-survival groups than in the rats with longer follow-up. However, no significant effects of EGb 761 treatment were observed on these parameters.

Discussion

In this study, we produced two types of cortical hemiplegia, one a mechanical lesion, and the other mechanical and chemical. In both, we were able to detect a benefi-
cial effect of EGb 761 on a motor task involving coordinated walking. This effect was more apparent in the motor-cortex aspiration group, where both short- and long-survival groups receiving EGb 761 performed better than the untreated rats. Although exhibiting a similar recovery trend during the first 3 d after injury, the GABA-infusion groups differed after that time and only the 7-d EGb 761-treated rats continued their progression towards a better recovery. Those under drug treatment for 30 d showed a profile similar to that of the untreated rats.

The aspiration group under EGb 761 had a salient functional recovery during the first 8 d of drug treatment, with motor performance comparable to that exhibited by the sham group. The GABA-infusion group did not show this behavior. Recovery in the latter group was slower and only became comparable to the sham group by the 20th d of treatment. Thus, EGb 761 effects clearly distinguish between different types of hemiplegia, suggesting a mechanism of action relatively independent from GABAergic processes.

The beneficial behavioral effects of EGb 761 have also been reported after frontal cortex lesions (Attella et al, 1989). In that study, EGb 761 decreased the hyperactivity induced by the cortical lesions and improved retention in a delayed-spatial alternation task. Those authors also reported that EGb 761-treated rats had smaller ventricles than untreated rats. We have confirmed this observation using a different model of cortical deficit.

It has been reported that acute oral administration of EGb 761 to young adult humans has a stabilizing effect on the EEG, as shown by an enhanced occurrence and persistence of the α rhythm. The EEG effects of EGb 761 are more conspicuous in aged patients, where slow activities in the δ band are diminished, with an increase in the presence and stability of α rhythms (see Pidoux, 1986). Our study involved chronic administration of EGb 761 to young adult rats, where we were unable to detect a significant effect of EGb 761 on cortical electrogenesis, as explored by EEG power spectral analysis. Although quite sensitive, spectral analysis is subjected to important variations due to changes in arousal levels, particularly in rodents habituated to laboratory conditions.

The lack of anticonvulsant effects of EGb 761 against the GWS is perhaps not surprising, as this epileptogenic syndrome is extraordinarily resistant to well-known anticonvulsant medications (eg, diazepam, barbiturates, phenytoin, carbamazepine, progabide), at least during the 1st d of epileptic activity. At present, both Naquet’s group at the CNRS in Gif-sur-Yvette and our group are investigating GWS refractoriness to antiepileptic agents.

Histological results showed no definite effects of EGb 761 treatment and we could not demonstrate significant differences between EGb 761-treated and untreated rats. Similar results were obtained in the study by Attella et al (1989).

The distant histological changes observed at the thalamic level are similar to those we reported in rats undergoing a GWS (Brailowsky et al, 1988), and were recently confirmed by Ross and Ebner (1990). These authors showed that either epileptogenic (kainic acid injection) or ablation lesions in somatosensory cortex induce comparable thalamic changes, demonstrated by GFAP staining. Thus, both excessive depression (excitotoxicity) and excessive excitation (inhibitotoxicity) can lead to cell death (Will et al, 1988).
In conclusion, EGB 761 treatment had a beneficial effect on functional recovery after motor-cortex lesions, without any apparent electrical or histological correlate, and with no toxic effects after chronic treatment.

These results justify further research on the effects of EGB 761 in brain pathology, in particular to search for the active principle(s) involved in the beneficial actions of the extract.

Acknowledgments

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References