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Effect of Aminophylline on Cerebral Infarction in the Mongolian Gerbil

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SUMMARY The effects of aminophylline in Mongolian gerbils subjected to unilateral carotid ligation were studied. The drug was given in varying intraperitoneal doses at varying postoperative intervals and the animals observed for 5 days for clinical signs of stroke. Doses of 100 mg per kg caused early death and were discontinued. Doses of 50 mg per kg had no significant effect on morbidity, mortality, time until death, stroke incidence or lesion size, as compared to saline given as a control. Doses of 80 mg per kg caused a higher mortality, higher morbidity, and a shorter interval to death, but a smaller infarct. Thus, aminophylline did not have a protective effect against stroke in gerbils and was actually detrimental during the first 16 hours following the carotid ligation.

INTRACEREBRAL microcirculation is depressed following cerebral infarction; therefore, one treatment of stroke is the augmentation of blood flow to the ischemic cerebral tissue. Vasodilatory drugs have not been successful in this regard, possibly because the blood-brain barrier has a higher drug threshold than peripheral vessels, and the more widely dilated peripheral vessels receive the greater proportion of available oxygenated blood. There has been conflicting evidence on the effects of aminophylline on cerebral blood flow. Aminophylline was originally thought to be a cerebral vasodilator, and it has been shown to reverse acute cerebral vasospasm following subarachnoid hemorrhage in monkeys and cats. However, more recently, cerebral vasoconstriction has been demonstrated following aminophylline therapy in human subjects. Some authors have observed such vasoconstriction only in healthy cortical tissue; thus, by an “inverse steal,” it is possible that blood flow could be increased in the diseased cortical areas. Aminophylline also increases ventilation and cardiac output, which may also be beneficial to ischemic cerebral areas.

These findings led us to study the effectiveness of aminophylline in the treatment of experimental stroke in the gerbil. The Mongolian gerbil (Meriones unguiculatus) was used as the experimental model because a high incidence of ipsilateral cerebral infarction is seen following ligation of one common carotid artery, probably due to the presence, in a large number of these animals, of an anomalous circle of Willis.

Methods

Two hundred young adult gerbils weighing from 30 to 40 gm were used, 20 serving as nonoperated controls, 180 being anesthetized with an intraperitoneal injection of ketamine (44 mg per kg) and undergoing a midline ventral cervical incision with exposure of the...
left common carotid artery. In 160 of the latter, the artery was doubly ligated and transected; in the remaining 20, the artery was isolated but not ligated. Immediately after operation, the 180 animals received 0.7 ml of 1% trypan blue injected intraperitoneally. The same dose of trypan blue was given to the 20 non-operated control animals.

Half the animals in each of the 3 groups were given 50, 80 or 100 mg per kg of aminophylline intraperitoneally at 1 hour after ligation or, in the case of the unoperated control animals, 1 hour after the start of the experiment. The 50 and 100 mg per kg doses were repeated every 8 hours thereafter for 3 days. The remaining animals received saline intraperitoneally on the same schedules. These regimens were administered in a double-blind manner. All animals were evaluated for mortality and signs of morbidity 1 hour after treatment, every 8 hours for 3 days, and then once daily for the next 2 days. Clinical signs of stroke included ptosis, eye fixed open, paucity of movements, obtundation (slowed) movement, cocked head, circling behavior, seizure, and coma (nonresponsiveness to external stimuli). A morbidity rating was assigned to each gerbil at each examination on the basis of these signs.

On the fifth postoperative day, surviving gerbils were killed. Brains from all animals were removed, examined for the presence of trypan blue stain (indicative of cerebral infarction), fixed in 10% formalin, and examined macroscopically. The brains of gerbils dying before the first morbidity check at 1 hour were examined microscopically for infarction, since there may not have been sufficient time for the trypan blue to leak into the infarcted nervous tissue.

Differences in the proportion of infarcts among regimens were evaluated using the chi-square test. Life tables were constructed to indicate the cumulative probability of dying by the time of each evaluation, and differences among regimens were tested by Fisher's Exact Test. Differences in time to death, morbidity rating (at each examination) and lesion size among regimens were examined using the Mann Whitney U-Test.

**Results**

When 100 mg per kg doses of aminophylline were used, deaths occurred before the 8-hour check.

There were no significant differences in outcome among the saline-treated groups; therefore, these groups were combined and reported as a single group.

The percentages of gerbils developing infarcts (saline — 38%, 50 mg per kg aminophylline — 50%, and 80 mg per kg aminophylline — 40%) were not significantly different ($p > 0.05$).

Results from life tables for the 3 groups are presented in the figure. Fisher's Exact Test showed no significant differences between the 50 mg per kg and the 80 mg per kg aminophylline-treated groups. The saline-treated group proved to have a significantly ($p < 0.05$) lower probability of dying than the 50 mg per kg aminophylline-treated gerbils at the eight-hour and 16-hour observation times. The saline-treated group also had a significantly ($p < 0.05$) lower probability of dying than the 80 mg per kg aminophylline-treated group at the one-hour, 16-hour and 24-hour observation times.

The saline-treated group had a significantly ($p < 0.02$) longer time until death than the 80 mg per kg aminophylline-treated group, but there were no significant differences in the time until death between the 50 mg per kg and 80 mg per kg aminophylline-treated groups or between the 50 mg per kg aminophylline-treated group and the saline-treated group. There were no significant differences in morbidity ratings at any observation period.

Lesion size was estimated to be 19.6% of the total cerebral tissue in the gerbils treated with 80 mg per kg aminophylline and to be 36% in the gerbils treated with saline. This difference was significant at the $p < 0.05$ level.

**Discussion**

Kogure has shown pretreatment with aminophylline to be beneficial in experimental stroke in rats, decreasing fluid accumulation and mortality. In order to simulate more accurately the clinical situation, we delayed treatment in our study until 1 hour after ligation.

Our results showed no indication that aminophylline prevented stroke in gerbils with left common artery ligation.
carotid ligation, but did show a significantly increased morbidity and mortality when infarction occurred. Apparently post-ligation treatment with aminophylline was detrimental during the first 24 hours of stroke.

After the first 24 hours, the rate of death in the 80 mg per kg aminophylline-treated group was similar to that in the saline-treated group. The average lesion size was 19.6% of the total cerebral tissue when 80 mg per kg of aminophylline was given, as opposed to 36% when saline was given. This apparent decrease in the size of the infarct with treatment may have been due to the more rapid onset of death in the 80 mg per kg aminophylline-treated gerbils, there thus being less time for the lesion to progress and for trypan blue to stain fully the infarcted area before death.

Ketamine was chosen as the anesthetic since pentobarbital has been shown to protect gerbils from stroke during the onset of ischemia. Nevertheless, as compared to pentobarbital studies conducted in this laboratory, the present study showed no significant change in the incidence of stroke when ketamine rather than pentobarbital was used.

We conclude that aminophylline administered post ligation has no value in this model. In fact, it appears that aminophylline can be harmful in gerbils, since both the 50 mg and 80 mg per kg aminophylline-treated groups had a high probability of dying during the first 24 hours. Also, animals in the 80 mg per kg aminophylline-treated group had a shorter time until death. This detrimental effect may be due to the high serum levels that accumulate because gerbils highly concentrate urine. Possibly, the lack of recanalization after ligation in this model and the fact that treatment was probably started postinfarction may have prevented the benefits of aminophylline reported in other models.

References
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