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Comparison of the Effects of Vinpocetine, Vincamine, Phenytoin, and Cinnarizine in a Rat Model of Cerebral Ischemia

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ABSTRACT

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Vinpocetine is an eburnamenine derivative reported to protect the brain from ischemia, both in experimental animals and in man. Its effects have been directly compared to those of vincamine, phenytoin, and cinnarizine in the Fisher rat following bilateral carotid artery occlusion (BCAO). Upon acute b.i.d. administration (25–100 mg/kg i.p.), both vinpocetine and vincamine significantly increased latency to ischemic convulsion in a dose-related manner, but neither drug significantly affected survival time. Neither phenytoin nor cinnarizine (25–100 mg/kg, b.i.d.) significantly altered latency to convulsions or survival time. After daily dosing for 5 days, vinpocetine, but none of the other drugs, caused a dose-related increase in the latency to ischemic convulsion. Vinpocetine's effects occurred at lower doses (25 and 50 mg/kg/day) after subchronic administration than after acute administration. Vinpocetine (25 mg/kg/day) and cinnarizine (100 mg/kg/day) also increased survival time. These results are consistent with other experimental and clinical studies demonstrating a protective effect of vinpocetine against cerebral ischemia and further show that vinpocetine's activity is maintained during repeated administration.

Key words: seizures, survival, stroke, subchronic administration

INTRODUCTION

Vinpocetine is an eburnamenine derivative reported to have beneficial effects in the treatment of cerebral ischemia [Kalvach et al., 1982; Otomo et al., 1985; Tamaki et al., 1985]. The goal of the present experiment was to compare the effects of vinpocetine with those of

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three other drugs (vincamine, cinnarizine, and phenytoin) in an animal model of cerebral ischemia. Vincamine, like vinpocetine, is an eburnamenine derivative found to be useful in treating the acute phase of stroke [Dekoninck et al., 1978; Thiery et al., 1979]. Cinnarizine is a vasodilator reported to act by blocking calcium entry [Van Neuten and Janssen, 1973] and to improve neurologic symptoms in patients with chronic cerebral ischemia [Passeri, 1978]. Phenytoin is well known as an anticonvulsant drug [Rall and Schleifer, 1980], but it has also been reported to protect against cerebral ischemia in patients suffering cardiac arrest [Aldrete et al., 1981]. Phenytoin was included in the present study because convulsions subsequent to cerebral ischemia were a prominent manifestation of the model used here.

The animal model used to assess drug effects was bilateral carotid artery occlusion (BCAO) in the Fisher (F-344) rat [Payan et al., 1965]. BCAO causes cerebral ischemia in this strain of rat, as evidenced by the fact that a temporary stoppage of carotid blood flow for 60 or 90 min results in a learning impairment [King, 1983] and in necrotic and edematous changes in brain when examined histologically (King, unpublished observations). In the study reported here drugs were tested, following acute and subchronic administration, for their activities in delaying the onset of ischemic convulsions and death resulting from BCAO. Some of these results have been previously reported in abstract form [King et al., 1985].

MATERIALS AND METHODS

Animals

Male F-344 rats were obtained from Charles River Breeding Labs (Wilmington, MA) and kept four per cage for at least 1 week prior to treatment, with food and water available ad libitum. The rats weighed between 182 and 230 g at the start of the experiment.

Drugs

Vinpocetine (Gideon Richter, Budapest, Hungary) and vincamine (Sigma, St. Louis, MO) were dissolved in 0.1 N HCl, and the pH was adjusted to 2.9 with NaOH. Cinnarizine (Janssen, Beerse, Belgium) and phenytoin (Warner-Lambert, Ann Arbor, MI) were suspended in 0.2% (v/v) Tween-80 in distilled water. All drugs, or their appropriate vehicles, were injected i.p. in a volume of 3 ml/kg.

Procedure

Rats were operated on in groups of ten to 12, beginning at 07:00. Anesthesia was induced with halothane in 95% O_2, 5% CO_2. The common carotid arteries were exposed bilaterally and carefully dissected free of surrounding connective tissue and nerve fibers. Two 3–0 sutures, separated by 2–3 mm, were tied tightly around each artery, and the arteries were severed between the sutures. The wound was closed with clips, and the rat was allowed to recover from the anesthesia. The operation required approximately 10 min. Animals dosed acutely (b.i.d.) were given the first injection immediately following BCAO and the second injection 6 hr later. Animals dosed subchronically prior to BCAO were injected once per day for 5 days in succession, with the last injection given immediately following BCAO. Postsurgery, the animals were divided equally among vehicle and drug treatment groups so that vehicle-treated animals and drug-treated animals were observed simultaneously. The animals that survived surgery were grouped three or four in an observation cage (37.5 × 25.4 × 22.9 cm), and a 60-W light bulb was illuminated 20 cm above the top of each cage to provide heat. The rats were observed for 12 hr: continuously for the first 7 hr and then every 15 min thereafter. The data recorded for each rat included latency to the first convulsive episode and latency to death. A total of 531 rats was used. Each vehicle control group had 17–28 rats, and each drug dose was tested in eight to 25 rats.

Statistics

The latency to the first convulsion and survival time were analyzed by the Gehan-Wilcoxon test for censored data [Gehan, 1965]. Of 531 rats, 42 (8%) survived longer than 12

hr; therefore, a maximum value of 12 was recorded for survival time. Thirty-six of these 42 animals had no convulsions within 12 hr, and a value of 12 was recorded for latency to first convulsion. Another 15 rats (3%) had no convulsions prior to death, and they were excluded from the calculation of latency to convulsion. The criteria for rejecting the null hypothesis was $P \leqslant 0.025$ for comparison of drug groups with their vehicle control.

RESULTS

The values for latency to convulsion and survival time of the vehicle control groups for vinpocetine, vincamine, phenytoin, and cinnarizine, are presented in Tables 1 and 2. No significant differences were found between the different control groups either dosed b.i.d. (Table 1) or subchronically (Table 2). Therefore, the data for drug groups is presented as a percentage of the respective control.

Effects of Acute Drug Administration

Immediately after recovery from anesthesia, most rats were quiescent. The first convulsion was invariably a violent running fit, which usually occurred between 1 and 4 hr postsurgery (Table 1). Both vinpocetine and vincamine caused a dose-related increase in the median latency to convulsion (Fig. 1), and this effect was statistically significant at 50 and 100 mg/g for both drugs. In comparison, neither phenytoin nor cinnarizine significantly affected the latency to convulsion (Fig. 1). None of the four drugs significantly altered the survival time following acute administration (Fig. 2).

Subchronic Drug Administration

Upon subchronic administration, vinpocetine increased the latency to convulsion in a dose-related manner, and this effect was statistically significant at 25 and 50 mg/kg/day (Fig. 3). None of the other three drugs tested altered latency to convulsion following daily administration for 5 days (Fig. 3).

Vinpocetine also prolonged the survival time. Although this effect of the drug was not dose-related, a significant effect was seen at 25 mg/kg/day (Fig. 4). Cinnarizine was the only other drug to increase the survival time following subchronic dosing. Cinnarizine increased

TABLE 1. Latency to First Convulsion and Survival Time for Acute Vehicle Control Groups

	Latency to convulsion (hr)			Survival time (hr)		
Vehicle control group	N	Median	Interquartile range	N	Median	Interquartile range
Vinpocetine	20	1.68	1.08–3.31	20	8.10	4.23–9.96
Vincamine	20	1.63	1.28–2.06	20	7.15	2.68–8.67
Phenytoin	27	1.43	1.26–2.42	27	4.72	2.33–8.64
Cinnarizine	17	2.02	1.75–2.73	17	6.90	3.97–10.58

TABLE 2. Latency to First Convulsion and Survival Time for Subchronic Vehicle Control Groups

	Latency to convulsion (hr)			Survival time (hr)		
Vehicle control group	N	Median	Interquartile range	N	Median	Interquartile range
Vinpocetine	26	1.78	1.22–2.81	26	6.11	3.56–8.78
Vincamine	26	1.50	1.08–2.31	28	5.02	3.10–8.01
Phenytoin	25	1.87	1.45–3.67	25	7.12	5.62–9.39
Cinnarizine	18	1.78	1.44–2.17	18	6.90	4.29–9.08

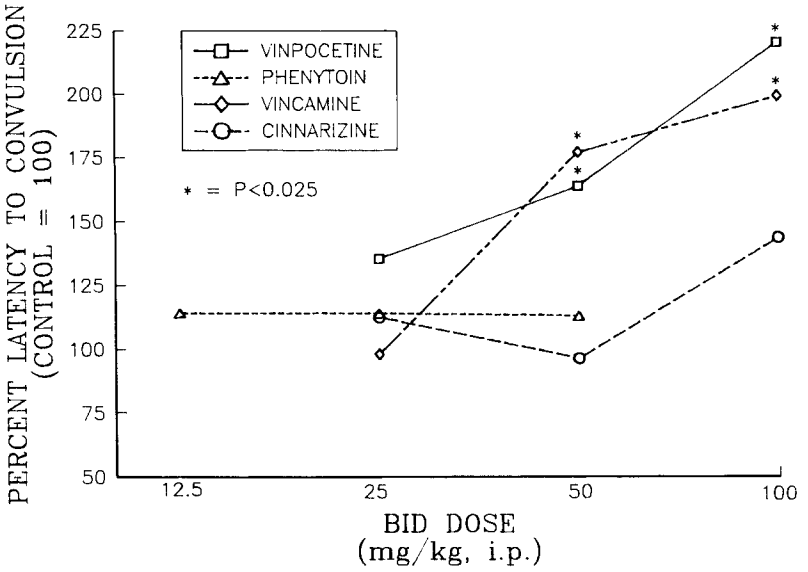

Fig. 1. Effects of acute b.i.d. administration of vinpocetine, vincamine, phenytoin, and cinnarizine on the latency to onset of ischemic convulsions following BCAO in the Fisher rat. Data presented are the median drug group scores expressed as a percentage of the respective vehicle control group score. Each point is based on the data of eight to 22 rats.

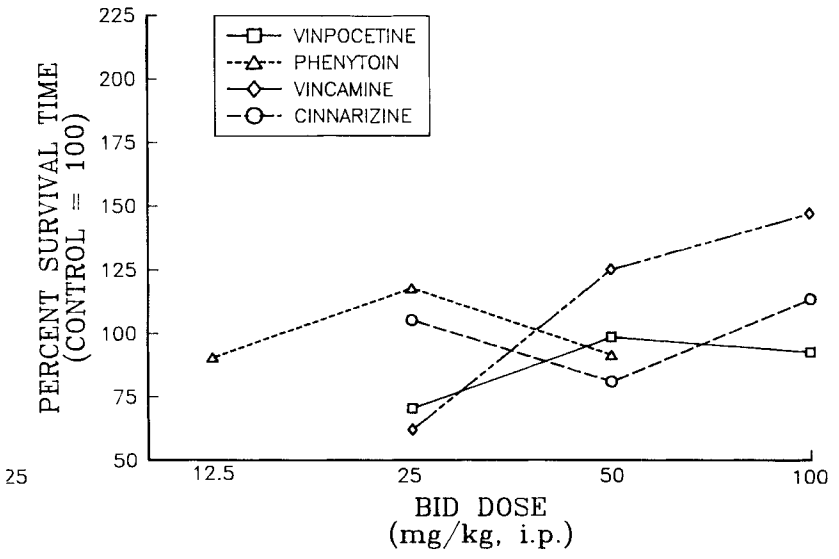

Fig. 2. Effects of acute b.i.d. administration of vinpocetine, vincamine, phenytoin, and cinnarizine on the median survival time following BCAO in the Fisher rat. Conventions for the presentation of data are the same as in Figure 1. Each point is based on the data of eight to 22 rats.

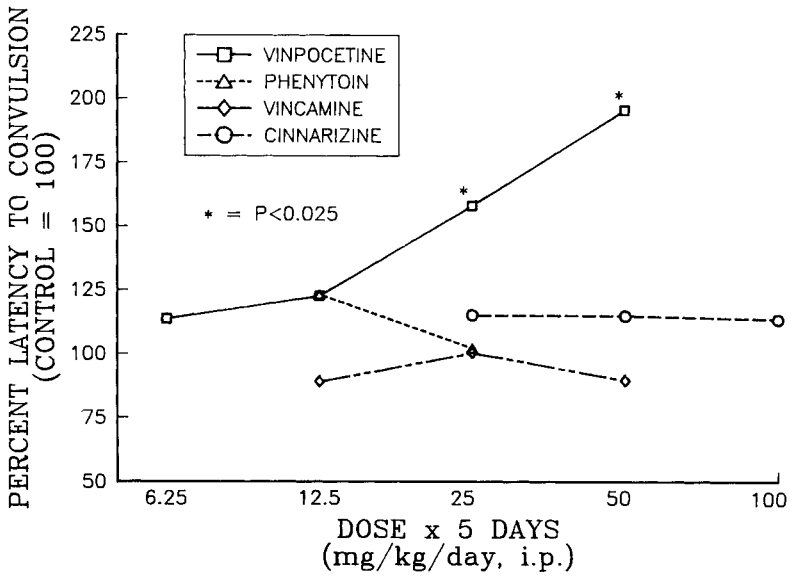

Fig. 3 Effects of once-daily administration (for 5 days) of vinpocetine, vincamine, phenytoin, and cinnarizine on the median latency to onset of ischemic convulsions following BCAO in the Fisher rat. Conventions for the presentation of data are the same as in Figure 1. Each point is based on the data of ten to 25 rats.

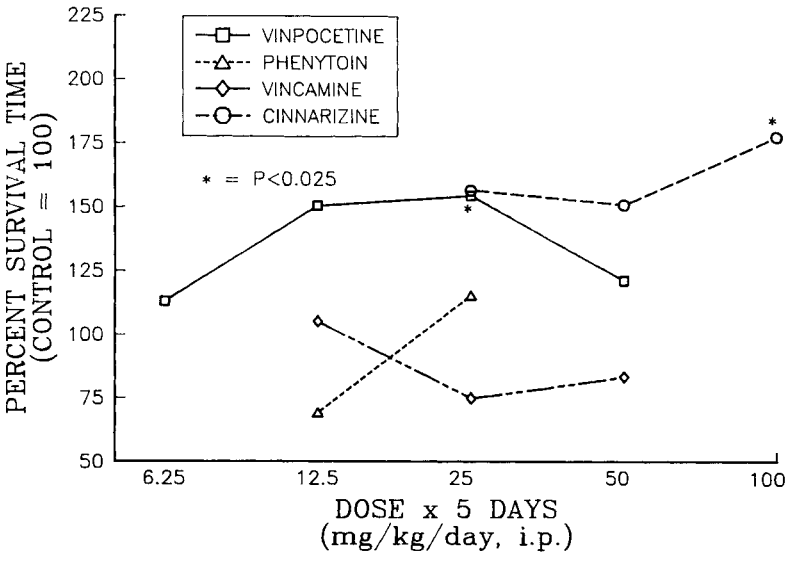

Fig. 4. Effects of once-daily administration (for 5 days) of vinpocetine, vincamine, phenytoin, and cinnarizine on the median survival time following BCAO in the Fisher rat. Conventions for the presentation of data are the same as in Figure 1. Each point is based on the data of ten to 25 rats.

this measure at all doses tested, but a statistically significant effect was observed only at the 100 mg/kg/day dose (Fig. 4).

DISCUSSION

In the Fisher rat cerebral ischemia model, vinpocetine given acutely or subchronically caused a dose-related increase in the latency to onset of ischemic convulsions. Furthermore, vinpocetine also increased survival time upon repeated application. The acute effects of vinpocetine on convulsion latency are consistent with similar observations by Kakihana et al. [1982] in a different rat strain. In comparison, vincamine, while demonstrating effects similar to those of vinpocetine upon acute administration, was ineffective when given subchronically. Neither phenytoin nor cinnarizine demonstrated any protective effects when given acutely. However, cinnarizine did increase the survival time following subchronic dosing.

The effect of cinnarizine to increase survival time following subchronic dosing is probably mediated by its Ca^{2+}entry-blocking activity [Van Neuten and Janssen, 1973]. Ca^{2+}-entry blockers improve tissue perfusion under conditions of low blood flow by preventing vasoconstriction that may be potentiated by anoxia [Vanhoutte, 1982]. Cinnarizine may also be acting by preventing excessive cellular Ca^{2+} uptake during ischemia. Ca^{2+} uptake by brain cells is known to be increased by ischemia [Siesjo, 1981], and elevated intracellular Ca^{2+} levels can be cytotoxic [Farber, 1981]. Further experiments measuring the effects of cinnarizine on cerebral blood flow and Ca^{2+} uptake during BCAO would be required to test these hypotheses.

Failure to observe any effects of cinnarizine on acute administration cannot be due to the choice of doses, since others have reported that cinnarizine (30 mg/kg) protects rats from hypoxia induced by sodium nitrite [Milanova et al., 1983] and lengthens the delay to disappearance of the cortical EEG in rats made anoxic [Karasawa et al., 1982].

It is not known whether vincamine's failure to affect convulsion latency following subchronic dosing represents drug tolerance. Loss of therapeutic effect on repeated dosing with vincamine is not reported elsewhere; however, the study reported here is the only instance in which vincamine's acute and subchronic effects have been compared in the same model. Ritschel et al. [1985] found that continuous infusion of vincamine, beginning 7 days prior to surgery, significantly increased the number of gerbils surviving unilateral carotid artery occlusion and reduced infarct size in surviving animals. Differences between our results and those of Ritschel et al. [1985] with regard to the efficacy of subchronically administered vincamine are likely the result of differences in animal species, severity of ischemia, and method of drug delivery.

Phenytoin, which is effective in suppressing epileptic seizures in man [Rall and Schleifer, 1980] and electroshock-induced seizures in rats [Swinyard et al., 1952], had no effect on latency to convulsion even at acute doses three to six times the ED_{50} for suppressing electroshock seizures in rats. Therefore, the mechanism for production of seizure activity in this model of cerebral ischemia may be unrelated to other epileptic phenomena.

These results further demonstrate the protective effects of vinpocetine in cerebral ischemia and suggest that the therapeutic effect seen in man [Kalvach et al., 1982; Otomo et al., 1985; Tamaki et al., 1985] will be sustained upon repeated administration.

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REFERENCES

Aldrete, J.A., Romo-Salas, F., Mazzia, V.D.B., and Tan, S.L.: Phenytoin for brain resuscitation after cardiac arrest: An uncontrolled clinical trial. Crit. Care Med. **9:**474–477, 1981.

Dekoninck, W.J., Joquet, P., Jacquy, J., and Henriett, M.: Comparative study of the clinical effects of vincamine + glycerol + placebo in the acute phase of stroke. Arzneim Forsch. **28:**1654–1657, 1978.

Farber, J.L.: The role of calcium in cell death. Life Sci. **29:**1289–1295, 1981.

Gehan, E.A.: A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. J. Biometrika **52:**203–223, 1965.

Kakihana, M., Suno, M., Shibota, M., Hamajo, K., and Nagaoka, A.: Protective effect of vinpocetine on experimental brain ischemia. Folia Pharmacol. JP. **80:**225–228, 1982.

Kalvach, P., Bauer, J., and Sagova, V.: Cavinton in the treatment of cerebrovascular disorders. C.S. Neurol. Neurochir. **45/78:**380–385, 1982.

Karasawa, A., Kumada, Y., Yamada, K., Shuto, K., and Nakamizo, N.: Protective effect of flunarizine against cerebral hypoxia-anoxia in mice and rats. J. Pharm. Dyn. **5:**295–300, 1982.

King, G.A.: The effect of carotid occlusion on the passive avoidance response in the F-344 rat. Soc. Neurosci. Abstr. **9:**643, 1983.

King, G.A., Narcavage, D., Romanski, L., Hannig, D., and Shieh, A.: Effects of vinpocetine in a rat model of cerebral ischemia. Soc. Neurosci. Abstr. **11:**433, 1985.

Milanova, D., Nikolov, R., and Nikolova, M.: Study on the anti-hypoxic effect of some drugs used in the pharmacotherapy of cerebrovascular disease. Methods Find. Exp. Clin. Pharmacol. **5:**607–612, 1983.

Otomo, E., Atarashi, J., Araki, G., et al.: Comparison of vinpocetine with ifenprodil and dihydroergotoxine mesylate treatment and results of long-term treatment with vinpocetine. Curr. Ther. Res. **37:**811–821, 1985.

Passeri, M.: Therapy of chronic consequences of brain ischemia. Eur. Neurol. (Suppl. 1) **17:**150–158, 1978.

Payan, H.M., Levine, S., and Strebel, R.: Effects of cerebral ischemia in various strains of rats. Proc. Soc. Exp. Biol. Med. **120:**208–209, 1965.

Rall, T.W., and Schleifer, L.S.: Drugs effective in the therapy of the epilepsies. In A.G. Goodman, L.S. Goodman, and A. Gilman (eds.): "The Pharmacological Basis of Therapeutics." New York: MacMillan Publishing Co., 1980, pp. 448–474.

Ritschel, W.A., Mandybur, T.I., Gummich, K.W., Vorhees, C.V., and Means, E.D.: Experimental cerebral infarction in mongolian gerbils: Effects of vincamine on lesion size, survival and behavior. Res. Commun. Chem. Pathol. **48:**221–242, 1985.

Siesjo, B.K.: Cell damage in the brain: A speculative synthesis. J. Cereb Blood. Flow Metabol. **1:**155–185, 1981.

Swinyard, E.A., Brown, W.D., and Goodman, L.S.: Comparative assays of antiepileptic drugs in mice and rats. J. Pharmacol. Exp. Ther. **106:**319–330, 1952.

Tamaki, N., Tadaki, K., and Matsumoto, S.: Effect of vinpocetine on cerebral blood flow in patients with cerebrovascular disorders. Adv. Ther. **2:**53–59, 1985.

Thiery, E., Otte, G., and Vander Eecken, H.: Comparative study of the clinical effect of vincamine versus papaverine given parenterally in the acute phase of stroke. Arzneim Forsch. **29:**571–574, 1979.

Vanhoutte, P.M.: Cinnarizine, flunarizine, lidoflazine. In T. Godfraind, A. Albertini, and R. Paoletti (eds): "Calcium Modulators," New York: Elsevier Biomedical Press, 1982, pp. 351–362.

Van Neuten, J.M., and Janssen, P.A.J.: Comparative study of the effects of flunarizine and cinnarizine on smooth muscles and cardiac tissue. Arch. Int. Pharmacodyn. **204:**37–55, 1973.