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CEREBROPROTECTIVE EFFECT OF PIRACETAM: ACUTE AND CHRONIC ADMINISTRATIONS OF PIRACETAM DURING SHORT-TERM AND LONG-TERM, INCOMPLETE AND COMPLETE CEREBRAL ISCHEMIA

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ABSTRACT

Cerebroprotective effect of piracetam by complete and incomplete short-term and long-term cerebral ischemia models on the rat brains was studied. The animals were subjected to 9 min of cerebral ischemia by bilateral carotid artery ligation for incomplete short-term ischemia, and to 4 hours cerebral ischemia by the same method for incomplete long -term ischemia model. Complete cerebral ischemia was induced by clamping aortic arch for 9 min (at intervals) after the injection of 100 mg/kg single intraperitoneal dose of piracetam (pyrolidine acetamide) for the acute treatment, and after the injection of the same daily dose of piracetam throughout 3 weeks for the chronic treatment. This study showed that acute and chronic use were the most effective treatments in short-term incomplete models in preventing cerebral damage, and only its chronic use was found to be effective in long-term ischemia.

Key Words: Cerebral ischemia, piracetam, cerebroprotective effect, histology.

PIRASETAM'IN SEREBPROTEKTİF ETKİSİ: KISA VE UZUN SÜRELİ TAM VE YARIM SEREBRAL İSKEMİ SIRASINDA AKUT VE KRONİK PIRASETAM UYGULAMALARI

ÖZ

Bu çalışmada, sıçan beyinleri üzerinde uzun ve kısa süreli tam ve yarı iskemi modellerinde piracetam'ın serebroprotektif etkisi araştırılmıştır. Hayvanlar, kısa süreli yarı iskemi modelinde karotid arterin bilateral ligasyonu ile 9 dakika süreyle, uzun süreli yarı iskemi modelinde ise yine aynı yöntemle 4 saat süreyle serebral iskemiye uğratılmıştır. Tam serebral iskemi modelinde, aort kavsi 9 dakika aralıklı olarak klampe edilmiştir. Kronik tedavi için 3 hafta boyunca günlük 100mg/kg dozda piracetam uyguladıktan sonra, akut tedavi için 100mg/kg tek doz intraperitoneal piracetam verilmesinin hemen ardından iskemi modelleri uygulanmıştır. Çalışmamızın bulgularına göre, kısa süreli yarı iskemi modelinde de akut kullanımın etkili olmadığı ve uzun süreli iskemide yarı iskemi olması koşuluyla, kronik piracetam uygulamasının koruyucu etkisi bulunmuştur.

Anahtar Kelimeler: Serebral iskemi, pirasetam, serebroprotektif etki, histoloji.

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1.INTRODUCTION

Brain constitutes about 2% of total body weight, receives 1/6 th of the cardiac output and consumes about 20% of total body oxygen. It receives its blood supply from carotid and vertebrobasiller arteries. Since it does not have reserved oxygen, metabolic changes in neurons start in the early ischemic period and neural function loss and neuron damage starts within 3 to 10 minutes.It was reported that approksimately 200000 people die of stroke each year and it is the third common cause of death in United States (Chusid, 1979), (Price and Wilson, 1978). Although it is well known that there is not a specific drug to protect the brain against ischemia and to reduce the damage due to ischemia, many drugs with different properties are still being used for this purpose on clinical basis (Lantos et al., 1990). Piracetam, which has a chemical structure of 2-oxo-1-pyrollidone acetamide, has a structural similarity with gamma amino butyric acid (GABA), an inhibitory neurotransmitter in brain. It has been shown that piracetam increases tolerance to ischemia by slowing the creatine kinase activity (Lantos et al., 1990), (Pogady et al., 1977); keeps the venous level of lactate passed from arterial system high for a long period of time and increases ATP synthesis by increasing cerebral oxygenation and glucose utilization and thus acts as a neurotropic agent (Pogady et al., 1977), (Wilsher et al. 1987). It has been suggested that in cerebrovascular insufficiency conditions defined in cerebral trauma, narcotic abuse and senile psychopathy which causes brain tissue damage and function loss, piracetam causes clinical improvement by its effect on telencephalone. This effect is thought to be achieved by its protective and reparative function on neurones and thus increasing their diminished neuronal performance (Pogady et al., 1977), (Ernst, 1973). The cerebral protective effect of piracetam ,however, varies according to the type of ischemia and to the therapy protocols (Wilsher et al. 1987), (Nikolov et al., 1990) In this study the cerebroprotective effects of piracetam were tested in different ischemia models. Wistar rats, were chosen for experiments, because rats has the advantage that, compared to other species, its cerebral circulation more closely resembles that of man (Kaneko et al., 1985).

2.MATERIALS AND METHODS

In this study 40 adult Wistar rats, from both sexes, weighting between 150-200 gr. were used. Animals were divided into 10 groups, each consisting of 4 animals. Single dose of piracetam was given intraperitoneally (IP) on the day of ichemia to observe its acute effect and daily dose of 100 mg/kg was given for 3 weeks prior to ischemia for its chronic effect. Before ischemia, rats were anaesthetized with ketamine hydrochloride (Ketalar®) 125 mg/kg intramuscularly. Bilateral carotid arteries were ligated for incomplete and short term ischemia model for 9 minutes and for incomplete and long term ischemia model for 4 hours. Thorax was opened and the rats were ventilated through a tracheal catheter in total ischemia group. Archus aorta was clamped 9 times with consecutive 50 seconds ischemia and 10 seconds reperfusion periods. The study groups were outlined in Table 1.

Specimens taken from frontal cortex and basal ganglia were fixed in 4% glutaraldehyde in phosphate buffer, pH.7.2-7.4 for electron microscopic examination. Some other specimens taken from different parts of the brain by coronal sections were fixed in 10% neutral formaline for one week and mordanted in 25% potassium dichromate for 3 weeks. Paraffin blocks for light microscopic examination and araldite blocks for electron microscopic examination were prepared in usual manner. Paraffin sections (10 mm) were stained with haematoxylin-eosin and semi-thin araldite sections were stained with toluidine blue. Both were examined under light microscope. Thin araldite sections were examined by electronmicroscope.

3.RESULTS

Postischemic hyperemia and edema were prominent pathologic findings in both three negative control groups (Groups B, C, D) in comparison with the positive control group (Group A). Hyperemia and edema were more severe and diffuse in long term ischemic group (Groups C, D) than short-term and incomplete ishemic group (Group B). It was observed that the intensity of the degenerative changes in neurons, neuroganglia and intercellular components increased as long as the duration of ischemia was increased, and these effects was less severe in short-term incomplete ischemia group (Figure 1). Thrombus and haemorrhage were the prominent findings only in complete and long-term ischemia group (Group D).

The effect of piracetam on the changes of neurones, glial cells and intercellular components was dependent on the time and the completeness of the ischemia and the acute or chronic use of the drug (Groups E,F,G,H,I and J). It was observed that the acute and chronic use of piracetam prevented the damage of the brain tissue in short-term incomplete ischemia groups (Groups E,F) (Figure 2). It was observed that the acute use of piracetam did not have any influence on long term complete and incomplete groups (Groups G, I) (Figure 4). On the other hand, the chronic use of piracetam had partially preventive effect (Figure 3, 5) on the same models (Groups H, J).

Table 1. Experiment groups and piracetam applications.

Groups		Ischemia method	Ischemia time	Piracetam application
Group A	Positive contol group	--------	----------	--------
Group B	1.Negative control group Short-term incomplete ischemia	BCL	9 minutes	--------
Group C	2.Negative control group Long-term incomplete ischemia	BCL	4 hours	--------
Group D	3.Negative control group Long-term complete ischemia	AAC/VT	9 minutes 9x(50"+10")	--------
Group E	Acute piracetam application in Short-term incomplete ischemia	BCL	9 minutes	100mg/kg, IP, single dose
Group F	Chronic piracetam application in Short-term incomplete ischemia	BCL	9 minutes	Daily dose of 100mg/kg, IP,for three weeks
Group G	Acute piracetam application in Long-term incomplete ischemia	BCL	4 hours	100 mg/kg, IP, single dose
Group H	Chronic piracetam application in Long-term incomplete ischemia	BCL	4 hours	Daily dose of 100 mg/kg, IP, for three weeks
Group İ	Acute piracetam application in Long-term complete ischemia	AAC/VT	9 minutes 9x(50"+10")	100 mg/kg, IP, single dose
Group J	Chronic piracetam application in Long-term complete ischemia	AAC/VT	9 minutes 9x(50"+10")	Daily dose of 100 mg/kg, IP, for three weeks

AAC: Archus aorta clamping
VT: Ventilation via tracheostomia
IP: Intraperitoneal injection
9x(50"+10"): 9 times 50 seconds ischemia and 10 seconds reperfusion sessions.

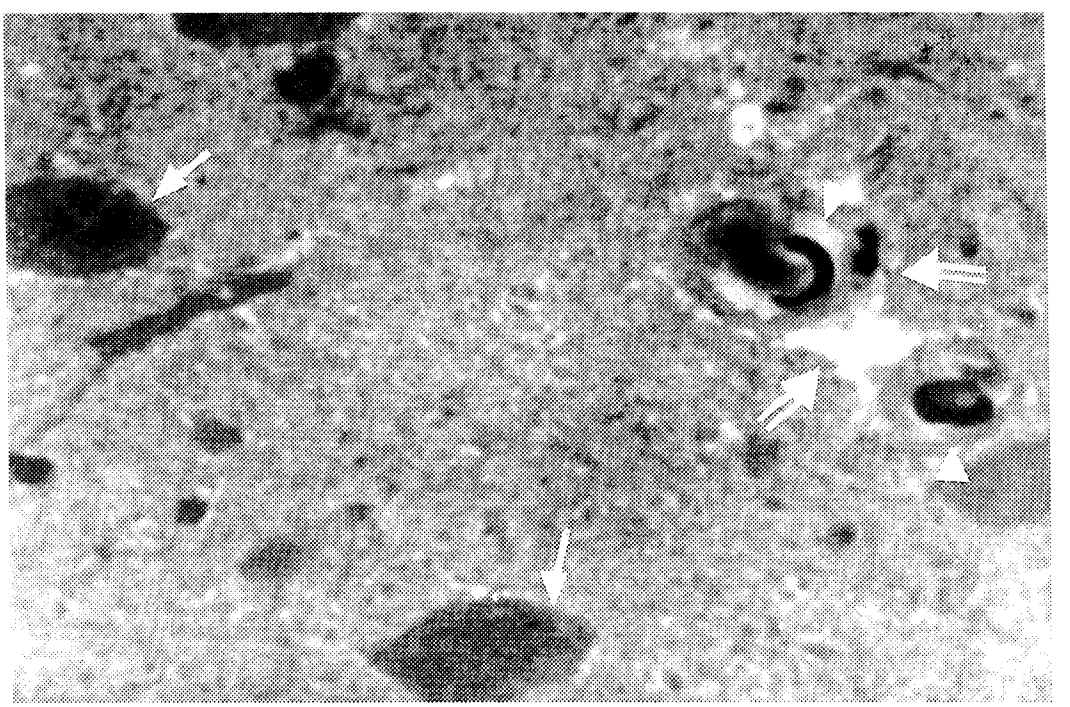

Figure 1. Minimal neuronal damages (Æ) and lower severity post-ischemic hyperemia(—) and edema (Ş) in short-term incom plete brain ischemia model (Group B). Toluidine Blue, X500.

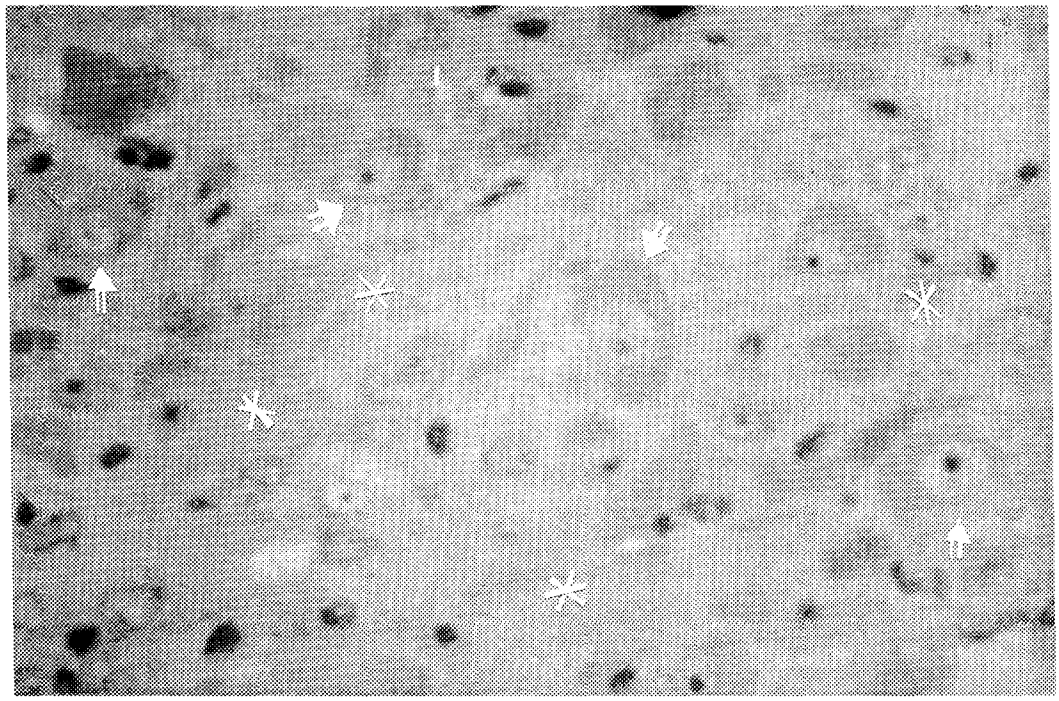

Figure 2. Complete protection after ischemic damage for neuronal (Ş) and intercellular components (*) in acute and chronic administration in short-term and incomplete ischemia (Group E and F). Toluidine Blue, X330.

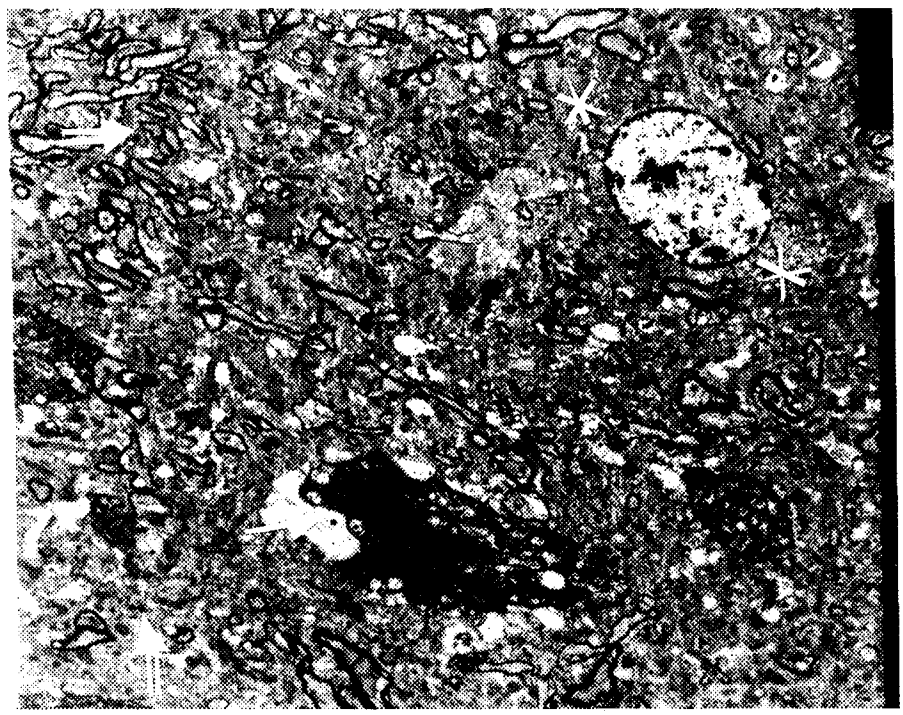

Figure 3. The partially protection appeared in long-term administration of piracetam in long-term incomplete ischemia model (Group H). Minimal neuronal (*) and neuroglial (→) damages and intercellular components (⇒) TEM, X 1800.

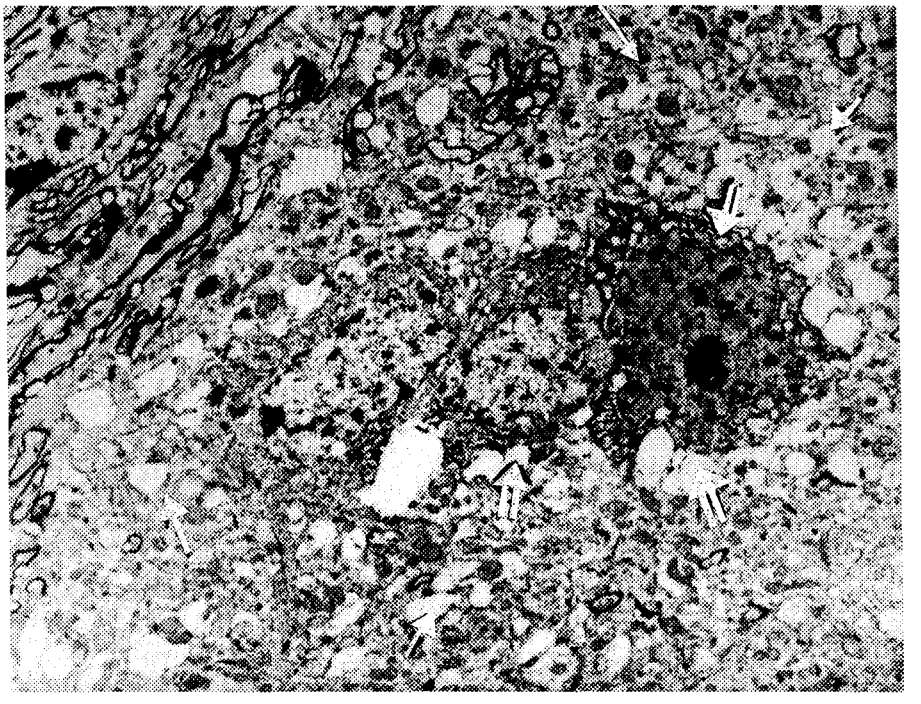

Figure 4. **The protection that was not observed in acute piracetam administration in long-term complete ischemia model (Group I). Intercellular (→) and intracellular (⇒) vacuolisations. TEM, X 1800.**

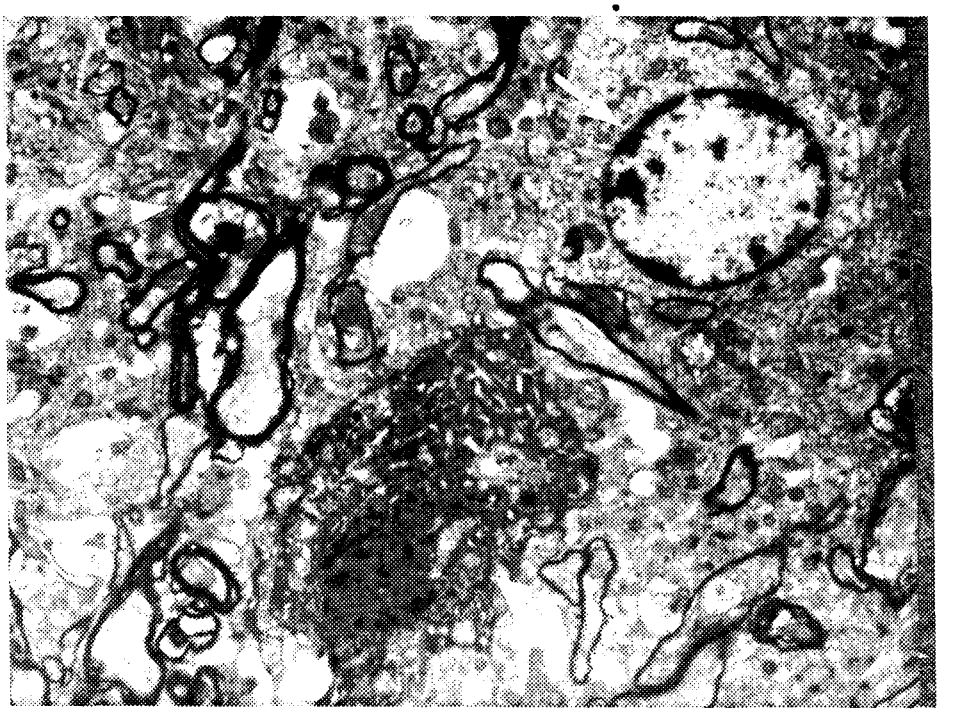

Figure 5. **Chronic piracetam administration in long-term complete ischemia model (Group J) partially prevented the neuronal (→) and neuroglial (⇒) damages and intercellular components (∇). TEM, X 2700.**

4.DISCUSSION

Since the brain tissue does not have regenerative potential, prophylaxis is much more important in unexpected brain damage.'Cerebral ishemia takes an important part in acute and chronic damage. There are a variety of conditions that cause ischemia; thus, treatment and prophylactic protocols differ according to the cause and the type of ischemia (Chusid, 1979), (Nikolov et al. 1990). Experimental studies showed that postischemic hyperemia developed after impairment of cerebral circulation and that accumulated strong vasodilator agents were responsible for the tissue damage. It is also observed that hyperemia developed even in short term ischemia and almost all parts of the brain were effected similarly in different ischemia models. Increased ischemia time causes accumulation of metabolic vasodilators and ischemia enhances (Todd et al., 1986a). Edema, an important complication of cerebral ischemia, causes transtentorial herniation and death, frequently. There is a direct relationship between the amount of fluid collection in brain tissue and the time and the degree of completeness of the ischemia. The degree of postischemic cerebral edema is more prominent after long-term ischemia(Todd et al., 1986a), (Todd et al., 1986b). In this setting, local and minor postischemic hyperemia and edema were observed in short term incomplete ischemia groups, whereas diffuse and major changes were observed in long term ischemia rats (Group C and D).Trombus and haemorrhage was also observed in long term complete ischemic rats (Group D). These findings were similar to the other studies which show the correlation between the time and the degree of the ischemia with neural changes and it also confirms the success of our ischemia model (Chusid, 1979), (Price and Wilson, 1978), (Todd et al., 1986a), (Todd et al., 1986b)

It is a known fact that abnormal secretion of glutamate and similar exitator neurotransmitters are responsible for the ischemic neuronal damage. Protein kinase C, cyclooxygenase, serotonin and free radicals play roles in these changes. Moreover, it was also shown that there is a close relation between the decreased ATP and increased lactate levels with the abnormal electroencephalography (EEG) findings; with the restored cerebral circulation, ATP production increases, lactate level decreases and EEG findings return to normal pattern (Kaneko et al., 1985), (Todd et al., 1986b). It is thought that the increase in the level of extracellular potassium ion in the ischemic brain tissue is a good indicator of cerebroprotective effect of the drugs. The comparative experiments done for this purpose showed that piracetam had a protective effect in short-term ichemia by increasing extracelluler potassium concentration and that the other agents were more effective in long-term ischemia group (Lantos et al., 1990). The observation of cerebroprotective effect of piracetam on short-term ischemia models (Group E,F) and the absence of similar effect on long-term ischemia models in acute use (Group G,I) was in accordance with the other studies in the literature. On the other hand, chronic piracetam therapy had beneficial effects on long-term ischemia groups (Group H,J). This finding is in contradiction with that of the other works which state the ineffectiveness of chronic piracetam therapy in long-term ischemia models. It should be pointed out that in all short and long-term ischemia models, acute piracetam treatment was not found to have any beneficial effect, that's why these authors have seen the results only in acute treatment. Piracetam was compared with flunarizine and with some other drugs in various ischemia models and it was found that piracetam provided a longer survival time than the other agents in chronic hypoxia states. In incomplete ischemia model done by bilateral carotid ligation, piracetam had a similar protective effect with flunarizine and cinnarizine, but was superior than the other agents. On the other hand, protection of piracetam, like the other agents was less than flunarizine in hypobaric hypoxia, complete ischemia with decapitation and anoxic hypoxia states (Nikolov et al., 1990). It was suggested that the contradictory beneficial effect of piracetam in cerebral ischemia models were due to the diversity of therapy protocols in the same ischemia model (Lantos et al., 1990), (Nikolov et al. 1990).

In this study, acute and chronic therapy protocols were used in both complete and incomplete, short and long-term ischemia models. The different clinical observations in different studies give us the impression that various mechanisms might be responsible for cerebral protective effect of piracetam. The beneficial effect of chronic use of piracetam in various ischemia settings, supported its efficacy in prophylactic mechanisms.Further studies are needed to identify these mechanisms. In a multicentric study, the adverse effects of piracetam with the placebo groups showed similarities and thus it seems that its chronic use will be tolerable(Wilsher et al., 1987).

This study which was designed to investigate the protective effects of piracetam in cerebral ischemia showed that acute and chronic use was the most effective in short-term incomplete models to prevent cerebral damage. Only its chronic use could also be effective in long-term complete and incomplete ischemia. It is concluded that the chronic use of piracetam may be effective in stroke and the pathologies associated with cerebral ischemia, but further studies are still needed.

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