EFFECT OF CARBIDOPA AND DIMETHYLSULFOXIDE ON PERMEABILITY OF THE BLOOD–BRAIN BARRIER FOR ^{14}C-TYROSINE AND ^{14}C-DOPA IN RATS

S. A. Borisenko

UDC 612.824.1:612.388.064/.014.46.088.6

KEY WORDS: carbidopa; dimethylsulfoxide; blood–brain barrier; tyrosine; dopa.

The modern concept of the blood–brain barrier (BBB) [1] defines two principal morphological and functional components of the barrier: the membrane component (the wall of the endothelium of the cerebral microcapillaries), and the enzyme component (enzymes of the endothelium of the microcapillaries), which play a decisive role as regulators of the entry of physiologically active substances, including drugs, into the brain. Unlike certain other amino acids, which pass comparatively readily through the BBB and, in particular, such as tyrosine, the factor limiting the passage of dopa through the BBB is the highly active enzyme dopa decarboxylase, which is located not only in peripheral capillaries, but also in the endothelial wall of the cerebral microcapillaries [6]. One way of reducing dopa-decarboxylase activity in order to facilitate the entry of dopa into the brain for the treatment of dopamine-deficiency states is the use of the dopa-decarboxylase inhibitors carbidopa (alpha-methyl-dopa-hydrazine) and bencerazine, which are components of the commercial preparations Sinemet and Madopar, and which enable the therapeutic effect of dopa to be realized despite a fourfold reduction in its dose [7]. Another possible way, in principle, of increasing the passage of dopa into the brain is by action on the membrane component of BBB by means of substances leading to reversible "opening" of the barrier, which can be done with the aid of dimethylsulfoxide (DMSO) [2], which has the property of increasing permeability of BBB for tryptophan [3], adrenalin and noradrenalin [5], and also sucrose, insulin, and the therapeutic preparations vincristine and bleomycin [2].

We compared the effect of weakening the enzymic and membranous components of BBB on permeability of the barrier for labeled tyrosine and dopa administered peripherally.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 300-350 g. Carbidopa in doses of 40 and 80 mg/kg and DMSO in doses of 1.75, 3.5, and 7 g/kg (in the form of 20, 40, and 80% solutions respectively) were injected intraperitoneally 60 min before injection of the isotope, and in the case of their combined use, carbidopa was injected 75 min before the isotopes. ^{14}C-L-tyrosine and ^{14}C-L-dopa (specific radioactivity 492 and 5.4 mCi/mole respectively) were injected into the animals' femoral vein in a dose of 5 μCi, dissolved in 0.5 ml of physiological saline. Immediately after injection of the indicator, the cerebral vessels were washed free from blood with 60 ml of physiological saline, heated to 36°C, and injected through the left ventricle after preliminary division of the right atrium. Pieces of brain tissue weighing 100 mg from the region of the cortex, cerebellum, hypothalamus, and medulla were homogenized in 0.3 ml of 0.6 M $HClO_4$ solution. The homogenate was centrifuged for 20 min at 10,000g and the supernatant was treated with 0.3 ml of 1.5 M $KHCO_3$. After recentrifugation 0.3 ml of the supernatant was added to 10 ml of "Aquasol" universal cocktail and the number of disintegrations recorded on an LKB liquid scintillation counter (Sweden). The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The initial permeability of BBB for labeled dopa in all the brain structures studied was 4-5 times less than permeability of the barrier for labeled tyrosine (Table 1). Carbidopa had no effect on permeability for ^{14}C-tyrosine but led to a significant, dose-dependent increase

Department of Neuropharmacology, Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 686-688, December, 1988. Original article submitted January 13, 1988.

 0007-4888/88/0012-1718$12.50

TABLE 1. Effect of Carbidopa and DMSO on Permeability of BBB for ^{14}C-Tyrosine (Ty) and ^{14}C-Dopa

Experimental conditions	Radioactivity in brain structures (in cpm) in supernatant from 100 mg tissue			
	cortex	hypothalamus	medulla	cerebellum
Control - physiological saline intraperitoneally				
Ty	543±24 (12)	485±21 (12)	463±32 (12)	531±46 (12)
Dopa	132±8 (6)	93±5 (6)	89±7 (6)	138±11 (6)
Carbidopa intraperitoneally:				
In dose 40 mg/kg				
Ty	*4 572±19 (5)	*4 530±27 (5)	*4493±22 (5)	*4548±24 (6)
Dopa	536±23 (6)	380±21 (6)	438±36 (6)	625±58 (6)
In dose 80 mg/kg				
Ty	*4 597±23 (5)	*4505±29 (5)	*4513±31 (5)	*4571±36 (5)
Dopa	682±41 (7)	574±32 (7)	550±26 (7)	706±49 (7)
DMSO intraperitoneally:				
In dose of 1.75 g/kg				
Ty	566±36 (6)	527±42 (6)	*530±38 (6)	**612±43 6)
Dopa	146±11 (5)	86±9 (5)	102±13 (6)	124±9 (5)
In dose of 3.5 g/kg				
Ty	***925±46 (6)	*4869±64 (6)	***779±64 (6)	***990±48 (6)
Dopa	***284±19 (6)	***212±14 (5)	**178±9 (6)	**216±20 (6)
In dose of 7 g/kg				
Ty	*4 1240±145 (5)	*41100±104 (5)	*41051±125 (5)	*41230±87 (5)
Dopa	*4 425±36 (6)	*4 418±21 (6)	*4 506±38 (6)	*4 544±41 (6)
Carbidopa + DMSO:				
In doses of 80 mg/kg and 1.75 g/kg				
Ty	*4551±27 (5)	*4497±23 (5)	*4522±25 (5)	576±34 (5)
Dopa	756±63 (7)	681±54 (6)	635±44 (7)	***672±50 (7)
In doses of 80 mg/kg and 3.5 g/kg				
Ty	***891±52 (6)	*4884±46 (6)	***712±58 (6)	***831±49 (6)
Dopa	*4821±74 (6)	*4702±57 (6)	*4791±62 (6)	*4730±46 (6)
In doses of 80 mg/kg and 7.0 g/kg				
Ty	*41192±112 (5)	***1024±93 (5)	*41103±145 (5)	***1078±103 (5)
Dopa	*41086±94 (5)	*4981±86 (5)	*4873±78 (5)	*4978±81 (5)

Legend. *p < 0.05, **p < 0.01, ***p < 0.002, *^{4}p < 0.001. Number of specimens shown in parentheses.

in passage of labeled dopa into the brain, confirming the existence of a well-marked enzymic barrier between the blood and the brain for dopa. That this barrier is located at the level of the endothelium of the cerebral microcapillaries is shown by data [6] on a marked reduction of dopa-decarboxylase activity under the influence of carbidopa, determined in the wall of the endothelium of the brain microcapillaries, without any appreciably change in activity of the enzymic in tissue of brain structures. These facts also point to the possibility, in principle, of selectively weakening the enzymic component of BBB by the use of the corresponding inhibitors, while preserving activity of this enzyme in the brain. DMSO, in a dose of 1.75 g/kg, led to a moderate increase in the permeability of BBB for ^{14}C-tyrosine in the medulla and cerebellum, but had no effect on permeability of the barrier for ^{14}C-dopa in all structures studied (Table 1). In higher doses DMSO led to a dose-dependent increase in permeability of BBB for both isotopes, although the effect was stronger in the case of labeled tyrosine. With the combined use of carbidopa and DMSO, the permeability of the BBB for ^{14}C-tyrosine was the same as when DMSO was used alone, but permeability for ^{14}C-dopa was significantly increased compared with the effect of administration of carbidopa or DMSO alone (Table 1). Even with the least dose of DMSO, against the background of carbidopa a significant increase was observed in permeability of the barrier in all the brain structures studied.

It can be concluded from the results that weakening of the BBB for dopa can be brought about both by weakening the enzymic component of the barrier with the aid of specific dopa-decarboxylase inhibitors and also by acting on the membrane component of the barrier by substances which have the ability to activate mechanisms of nonspecific transport (pinocytosis), which include DMSO [2]. Under these circumstances the two mechanisms can evidently operate simultaneously. Meanwhile, it will be evident that the nonspecific character of the action of DMSO can lead to an unselective increase in the entry of substances with widely different functions into the brain. However, the possibility likewise cannot be ruled out that it is this "universality" of DMSO with respect to increasing the permeability of biological membranes that lies at the basis of its effectiveness in certain diseases and, in particular, in cerebrovascular disturbances caused by an increase of intracranial pressure [4].

LITERATURE CITED

1. M. Bradbury, The Concept of a Blood–Brain Barrier, Wiley, Chichester (1979).
2. R. Broadwell, M. Salcman, and R. Kaplan, Science, 217, 164 (1982).
3. J. C. De La Torre, Experientia, 26, 1117 (1970).
4. J. C. De La Torre, Biological Actions and Medical Applications of Dimethylsulfoxide, New York (1983).
5. J. Hanig, M. Morrison, and S. Krop, J. Pharm. Pharmacol., 23, 386 (1971).
6. J. Hardebo, B. Flack, C. Owman, and E. Rosengren, Acta Physiol. Scand., 105, 453 (1979).
7. S. J. Rapoport, Blood–Brain Barrier in Physiology and Medicine [in Russian], New York (1976).

PREVENTION OF DISTURBANCES OF CARBOHYDRATE AND OXYGEN METABOLISM OF THE BRAIN AND DEVELOPMENT OF CEREBRAL EDEMA BY ISOTHIOBARBAMINE IN THE EARLY PERIOD AFTER INTRACEREBRAL HEMORRHAGE

M. B. Plotnikov, T. M. Plotnikova, T. V. Yakimova, and A. S. Saratikov

UDC 616.831-005.1-06:616.831-008.9-084:/615.31:547.854.5/-092.9

KEY WORDS: isothiobarbamine; intracerebral hemorrhage; cerebral edema; disturbances of carbohydrate metabolism.

The pathogenesis of the early posthypoxic period of intracerebral hemorrhage (ICH) is characterized by accumulation of lactate in the brain, with its discharge into the venous blood, and also by the onset and progressive development of edema and swelling of the brain [7, 8]. Lactacidosis of brain tissue is one of the foremost damaging factors in acute cerebrovascular disturbances; it stimulates autolysis, it severely restricts activity of enzymes of brain energy metabolism, and promotes the development of edema [5, 13, 14]. In patients with a cerebrovascular accident, computed tomography has revealed the presence of close correlation between the degree of lactacidosis of the CSF and the magnitude of the edema [9]. In view of the facts described above it is interesting to assess the effects of drugs with an antiacidotic action on the brain against the development of edema. Among substances which limit lactate formation in the hypoxic brain, the most important are sodium hydroxybutyrate and barbiturates [10, 12]. However, these drugs exert their antiacidotic action on brain tissue only in doses inducing anesthesia. Moreover, in severe forms of ischemia and hypoxia, the antihypoxic properties of sodium hydroxybutyrate are not exhibited [10]. The use of the thiobarbituric acid derivative isothiobarbamine, which has a protective action and greatly reduces lactate accumulation in the cerebral cortex under hypoxic conditions, would seem to be more promising [3, 4].

We studied the effect of isothiobarbamine on the blood supply to the brain and on the oxygen, glucose, and lactate utilization of the organ and compared them with the antiedematous effect of the compound in cats after ICH.

EXPERIMENTAL METHOD

Experiments were carried out on 17 anesthetized and 13 conscious cats weighing 2.5-3.5 kg. ICH was reproduced by the method in [4]. The total cerebral blood flow was recorded in anesthetized cats (pentobarbital, 40 mg/kg), by means of an RKÉ-2 flowmeter in both carotid arteries after ligation of all the higher branches up to the internal maxillary arteries; values of pO_2, pCO_2, and pH were measured in samples of cerebral arterial and venous blood (on a "Godart" gas analyzer), concentrations of glucose and lactate were determined [6], and the oxygen, glucose, and lactate consumption of the brain was calculated as the product of the total

Institute of Pharmacology, Tomsk Scientific Center, Academy of Medical Sciences of the USSR. Tomsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 688-690, December, 1988. Original article submitted May 5, 1988.

 0007-4888/88/0012-1720$12.50