Pharmaceutical Research, Vol. 4, No. 2, 1987

Report

Prostaglandin Derivative PGB_x Improves Neurologic Recovery After Ischemic Spinal Injury

Thomas P. Jacobs,[1,3] John M. Hallenbeck,[1] Thomas M. Devlin,[2] and Giora Z. Feuerstein[1]

Received July 18, 1986; accepted October 28, 1986

Neurological dysfunction resulting from an ischemic insult in the central nervous system (CNS) is believed to be an indirect outcome of impaired energy production. Recent studies suggest that prostaglandin B_x (PGB_x), an oligomer of PGB_1 and 15-keto-PGB_1, may be beneficial in protecting mitochondrial function after ischemic insults by preventing uncoupling of oxidative phosphorylation. In the present study, PGB_x had a significant influence on neurological outcome after ischemic injury in the rabbit spinal cord. These findings suggest that PGB_x may be beneficial in the treatment of CNS ischemic injuries.

KEY WORDS: ischemia; paralysis; prostaglandins; spinal cord.

INTRODUCTION

Neurological dysfunction resulting from an ischemic insult to central nervous system (CNS) tissue is believed to be an indirect outcome of severe depletion of cellular energy stores and impairment in energy production (1,2). Although the sequence of events responsible for the death of cells in CNS ischemia is poorly understood, it is generally accepted that irreversible cell injury occurs in association with plasma and mitochondrial membrane disruption (1). The inadequacy of mitochondria to resume normal function even after tissue reperfusion has been implicated as an important factor in the pathogenesis of irreversible cell injury (3–5).

Recent studies have suggested that prostaglandin B_x (PGB_x), an oligomer of PGB_1 and 15-keto-PGB_1, may be beneficial in protecting mitochondrial function after ischemic insults by preventing uncoupling of oxidative phosphorylation (6). Other studies suggest that PGB_x may function as an ionophore and maintain phosphorylation of ADP by preventing uncoupling through an energy-dependent Ca^{2+} sequestration mechanism (7). Therefore, we have postulated that PGB_x, acting to preserve mitochondrial energy production, may have a beneficial effect on motor and neurological consequences after ischemic injury to the CNS.

To test this hypothesis, we have used a spinal cord ischemia model in which neurological function can be monitored after focal spinal cord ischemia in the unanesthetized rabbit (8). In this model, complete paralysis is observed during the ischemic event, followed by partial recovery of function upon reperfusion. However, several hours after reperfusion, secondary deterioration of function occurs which appears permanent (9).

The present study was undertaken to determine if PGB_x can improve neurological outcome after ischemic injury to the rabbit spinal cord.

MATERIALS AND METHODS

New Zealand albino rabbits (2.0 ± 0.25 kg) were anesthetized with ketamine hydrochloride (50 mg/kg, im) and sodium pentobarbital (40 mg/kg, iv). Under aseptic conditions, a transperitoneal approach was made to expose the abdominal aorta, and polyvinylchloride tubing (0.75-mm o.d.) was placed around the aorta distal to the renal arteries. This tubing was threaded through plastic buttons (6.0 mm) that were placed dorsal and ventral to the aorta to produce a snare ligature. To prevent movement through the incision site, the ligature was passed through a vinyl guide tube (6.25-mm o.d.), which was secured to the abdominal muscles. A canvas jacket was placed around the animal to protect the incision site and the externally accessible ligature. Approximately 18 hr postsurgery, when the animals were awake, the aorta was occluded for 25 min by pulling on the snare ligature and clamping with a pair of hemostat forceps. The ligature was subsequently released and removed with the guide tube through the surgical site. A retaining ligature, which was earlier placed around the abdominal muscles, was then secured. Animals were randomly assigned to PGB_x or vehicle control treatment groups. PGB_x solubilized in distilled water (provided by Dr. T. M. Devlin), 2.0 mg/kg ($N = 16$), or vehicle ($N = 16$) was given as an iv bolus at 1, 3, 5, 7, 9, and 11 hr after reperfusion. Injections were given in a volume of 0.5 ml over a period of 60 sec, followed by a 0.5-ml saline flush through a lateral ear vein catheter. Hindlimb function was graded at hourly intervals

[1] Department of Neurology, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.

[2] Department of Biological Chemistry, Hahnemann Medical College, Philadelphia, Pennsylvania 19102.

[3] To whom correspondence should be addressed at Department of Neurology, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, Maryland 20814-4799.

0724-8741/87/0400-0130$05.00/0

Table I. Effect of PBG_x Treatment on Hindlimb Motor Function After Ischemic Spinal Injury

Hindlimb score	2 hr		4 hr		6 hr		8 hr		10 hr		12 hr		24 hr	
	Control	PGB_x*	Control	PGB_x*	Control	PGB_x*	Control	PGB_x*	Control	PGB_x*	Control	PGB_x*	Control	PGB_x**
5	—	25 (4)[a]	—	25 (4)	6 (1)	31 (5)	6 (1)	31 (5)	—	31 (5)	—	31 (5)	—	19 (3)
4	19 (3)	19 (3)	13 (2)	31 (5)	19 (3)	19 (3)	6 (1)	25 (4)	13 (2)	25 (4)	13 (2)	13 (2)	—	—
3	56 (9)	44 (7)	75 (12)	44 (7)	50 (8)	50 (8)	63 (10)	38 (6)	63 (10)	38 (6)	63 (10)	50 (8)	19 (3)	25 (4)
2	19 (3)	6 (1)	13 (2)	—	19 (3)	—	13 (2)	6 (1)	6 (1)	—	6 (1)	—	19 (3)	6 (1)
1	19 (3)	6 (1)	—	—	6 (1)	—	13 (2)	—	19 (3)	6 (1)	19 (3)	6 (1)	56 (9)	31 (5)
0	—	—	—	—	—	—	—	—	—	—	—	—	6 (1)	19 (3)

[a] Neurologic scores during the 12-hr reperfusion period (see Materials and Methods). Numbers represent the percentage with each hindlimb score followed by the number of animals in parentheses.

* Statistically significant at $P < 0.05$, Mann–Whitney U test.

** Statistically significant at $P < 0.05$, Fisher's exact probability test.

during the treatment period and 24 hr after reperfusion by an investigator unaware of the treatment group. A strict blinded evaluation protocol was kept throughout the experiment. The following ordinal grading scale was used: 0 = complete paralysis; 1 = minimal functional movement, severe paresis; 2 = functional movement, supports weight, unable to hop; 3 = hopping, markedly ataxic and paretic; 4 = hopping, slightly impaired; 5 = normal function.

Animals were also categorized as hoppers (score 3, 4, 5) or nonhoppers (score 0, 1, 2). Neurologic scores between the groups were analyzed by the Mann–Whitney U test. Frequency analysis of hopping and nonhopping function was performed by Fisher's exact probability test. A P value <0.05 was considered statistically significant.

RESULTS

Neurological scores between animals treated with PGB_x and vehicle controls were significantly different (Table I). Differences between the groups were observed as early as 2 hr postreperfusion and were sustained during the 12-hr treatment period (Mann–Whitney U test, each $P < 0.05$). During this period there were 20% more hopping animals in the treated group compared to controls. This beneficial effect of PGB_x was sustained at 24 hr, with 42% of the rabbits hopping in the PGB_x-treated group, compared to 19% hoppers in the control group. The frequency of hoppers in the PGB_x-treated group, 7 of 16 rabbits, was significantly higher compared with the control group, 3 of 16 rabbits (Fisher's exact probability test, $P = 0.03$).

DISCUSSION

In the present model, control animals exhibited substantial recovery of motor function within 4 hr after reperfusion of the spinal cord. However, 12–18 hr later, a secondary decline in function occurred, which appeared permanent. This study demonstrated that PGB_x prevented much of the secondary decline in motor function after ischemic spinal cord injury in the rabbit. It has been suggested that PGB_x may function by maintaining oxidative phosphorylation in damaged mitochondria and prevents degradation of the ATP/O_2 ratio. Studies suggest that the site of action may be the F_1F_0 ATPase in the inner mitochondrial membrane, where it may prevent uncoupling of phosphorylation of ADP and electron transport (10). This working hypothesis for PGB_x may help us to understand the significant pathophysiological factors involved in ischemia-related CNS injuries. However, it does not preclude other possible mechanisms of action of PGB_x. Other studies have suggested that PGB_x may function as an ionophore and maintain oxidative phosphorylation by preventing uncoupling by an energy-dependent Ca^{2+} sequestration mechanism (7).

The results of this study are comparable to those of other studies using the rabbit spinal cord ischemia model to test potential pharmacological therapies (e.g., naloxone, WIN 44-441-3) for ischemic injuries in the CNS (8,11). With this model, a consistent degree of postischemic hindlimb motor dysfunction is produced which can be assessed in the unanesthetized state. This makes the model particularly suitable for testing the efficacy of potential pharmacological therapies.

Studies have shown that the effects of PGB_x may be dose dependent and higher concentrations of PGB_x may inhibit phosphorylation of ADP (12). In the present study, only one dose was evaluated. Therefore, due to the potential therapeutic value of PGB_x treatment for ischemic injuries in the CNS, additional studies focusing on the pharmacokinetics and mechanism of action are warranted.

ACKNOWLEDGMENTS

This work was supported by the Office of Naval Research (Contract No. G19219) and the American Heart Association (Contract No. G19218). The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Department of Defense or the Uniformed Services University of the Health Sciences. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council DHEW Publ. No. (NIH) 78-23. We wish to thank Ms. Debbie Anderson and Ms. Sharon Nagle for their technical assistance and Ms. Wanda D. Patterson for preparing the manuscript.

REFERENCES

1. J. L. Farber, K. R. Chien, and S. Mittnacht. *Am. J. Pathol.* **102**:271–281 (1981).
2. B. K. Siesjo. *J. Neurosurg.* **60**:883–908 (1984).
3. L. Mela. In R. A. Cowley and B. F. Trump (eds.), *Pathophysiology of Shock, Anoxia and Ischemia,* Williams and Wilkins, Baltimore, 1982.
4. S. Rehncrona, L. Mela, and B. K. Siesjo. *Stroke* **10**:437–446 (1979).
5. W. A. Pulsinelli, J. B. Brierley, and F. Plum. *Ann. Neurol.* **11**:491–498 (1982).
6. R. J. Kolata and B. D. Polis. *Physiol. Chem. Phys.* **12**:545–550 (1980).
7. M. Kruger and J. Booyens. *S. Afr. Med. J.* **62**:855–858 (1982).
8. A. I. Faden and T. P. Jacobs. *Neurology* **35**:1311–1315 (1985).
9. T. P. Jacobs, E. Shohami, E. Baze, E. Burgard, J. M. Hallenbeck, and G. Feuerstein. Submitted for publication (1985).
10. D. K. Kruetter and T. M. Devlin. *Arch. Biochem. Biophys.* **221**:216–226 (1983).
11. A. I. Faden, T. P. Jacobs, M. T. Smith, and J. A. Zivin. *Eur. J. Pharmacol.* **103**:115–120 (1984).
12. B. D. Polis, E. Polis, and S. F. Kwong, *Proc. Natl. Acad. Sci.* **76**:1589–1602 (1979).