THE ROLE OF BIO-CATALYZER NO. 11 (BIO-NORMALIZER) IN SPINAL CORD ISCHEMIA AFTER AORTIC CROSS-CLAMPING: AN EXPERIMENTAL STUDY

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INTRODUCTION

Since the inception of surgical therapy for lesions of the thoracoabdominal aorta in the early 1950's, paraplegia or paraparesis has been reported as a frequent, devastating and unpredictable complication of otherwise successful surgical procedures. Reported paraplegia rate in thoracoabdominal aneurysm carries a morbidity as high as 30% of patients in a series. (1) Several contributory factors were implicated, such as prolonged aortic occlusion, hypotension, etc., but the complication is basically related to a permanent or transient ischemia of the spinal cord during cross clamping of the aorta. While it's true that ischemia will by itself ultimately produce tissue necrosis if it's long and severe enough, in some clinical situations, a substantial part of the injury should be more properly termed reperfusion or post-ischemic injury. That is, much of the injury may not occur during the actual period of hypoxia, but rather during the period when molecular oxygen is reintroduced to the tissue. (2) The oxidative stress, which is a major contributory factor in the etiology of cerebrospinal ischemia, is in part attributed to production of free radicals such as superoxide anion , hydroxyl , oxygen and carbon centered radicals, as well as hydrogen peroxide . Antioxidants such as Vit E, glutathione, ascorbate, superoxide dismutase (SOD), catalase, among other, as well as natural herbal medicines with antioxidative properties have been analyzed and studied for its protective

action against cerebrospinal ischemia in different experimental studies. Recently, Bio-catalyzer No. 11 (Bio-Normalyzer), a commercially available natural health food supplement produced from the fermentation of Carica papaya Linn. (a Phil. medicinal plant) ,some tropical herbal plants, and cereals from Japanese tropical and traditional foods, was shown to exhibit diverse and wide-ranging biological, physiological and therapeutic properties (3).

This study was undertaken to determine whether the neurologic outcome after temporary aortic occlusion in rabbits could be improved with peri-operative administration of Bio-Normalyzer, which is a free - radical scavenger.

We would also like to determine whether significant differences exist between the three groups of rabbits given different diet regimens in terms of the following parameters : A. Final neurologic outcome B. onset of flaccid paralysis after aortic occlusion C onset of sensory loss after aortic occlusion and D. initial recovery from flaccid paralysis after reperfusion. And finally, whether the onset of flaccid paralysis and sensory loss after aortic occlusion, or the onset of initial recovery from flaccid paralysis after reperfusion, can serve as predictors of the final neurological outcome.

ABSTRACT

In order to determine whether Bio-catalyzer no. 11 (Bio-normalyzer), a free radical scavenger, could prevent spinal cord ischemia, snare occlusion devices were surgically implanted in 30 male rabbits which were randomized into 3 groups according to diet regimens. They were given standard diets of pellets and water. In addition, Group B was given table sugar, and Group C, Bio-normalyzer. 48 hrs. after recovery, the aorta was occluded by tightening the snares for 60 minutes and then, released. In Group C, Bio-normalyzer was given preoperatively and continued for 5 days. The onset of flaccid paraplegia, sensory loss after occlusion and the onset of neurologic recovery after reperfusion were recorded. Using statistical analyses, the neurologic outcome in Group C was shown to be significantly better than in Group A & B at p value less than .05. Likewise, the onset of paralysis after aortic occlusion could serve as a predictor of the final neurologic recovery. This study indicates that oxygen-derived free radicals play a role in ischemia-reperfusion injury of the spinal cord and treatment with Bio-normalyzer can improve the final neurologic outcome following temporary occlusion in rabbits.

KEYWORDS: Bio-normalyzer

Spinal cord ischemia

Thoracoabdominal aortic aneurysm

MATERIALS AND METHODS

Thirty male rabbits weighing about 800 to 1,000 grams were used in the study. After inducing anesthesia by Ketamine HCl, a snare occlusion device using Prolene sutures was surgically implanted around the infrarenal aorta of each animal and tunneled into the subcutaneous tissue (left paravertebral line) . They were then randomized into 3 groups according to the diet regimens. **Group A** , or control group (n=10) had ad libitum to a standard diet of pellet and water diet. **Group B** or placebo group (n=10) was given pellets, water and table sugar daily and in **Group C** , pellets, water plus Bio-normalyzer at 0.1 g/kg/day . The dose of Bio-catalyzer No. 11 (Bio-normalyzer) used approximate the recommended dose in humans (3 to 9 g/day)(4). Forty eight hours after recovery, the infrarenal aorta was occluded in the awake animals by retrieving and tightening the snare for 60 minutes, after which it was then released. In Group C, Bio-Normalyzer was given 2 hours prior to aortic occlusion and continued for 5 days via gavage method. The onsets of complete flaccid paraplegia, loss of sensory function after occlusion and the initial recovery period after reperfusion were recorded. The animals were observed periodically up to 5 days for neurologic recovery. Grading was done independently using the modified Tarlov's criteria as follows : **Grade 0** -no movement of the lower extremities; **Grade 1**- minimal movement of the hind limb; **Grade 2**- good movement but unable to stand; **Grade 3**- able to stand but not hop normally; **Grade 4** - complete neurologic recovery (1). On the 5th day after aortic occlusion, all

animals were then sacrificed and their spinal cords removed for histologic examination of the lumbar sections.

RESULTS

All the animals were able to survive through the 5 days observation period. Occlusion of the infrarenal aorta in the awake rabbit caused complete flaccid paraplegia w/ a mean of 63 secs. in all animals (A = 60, B = 65, C = 64) Table 2 and the period of ischemia necessary to cause loss of sensory function was longer with a mean of 230 scs. (A = 137, B = 248, C = 306). Table 3 Following restoration of aortic blood flow, 23% (7/30) remained completely paraplegic (Grade O) while the remaining 77% were able to regain various grades of motor function (Gr 1-4) within a mean of 172 min. (A = 175, B = 196, C = 145) Table 4, after reperfusion. However, in 47% of the animals that recovered, neurologic function began to deteriorate starting 12-24 hours after aortic reperfusion to a paraplegic state (Grade 0, 1) within the next 5 days. Although some animals recovered neurologically within the control group, final paraplegic rate (Gr 0, 1) was 70% (7/10). In Group B, 80% (8/10) remained paraplegic while only 30% (3/10) was seen in the treated group (C) Table I.

To determine whether differences exist according to the final neurologic outcome, the Kruskal-Wallis one way analysis of variance was used. This was because the response variable was in the ordinal scale with final neurologic outcome being assigned grades from 0 to 4. The results indicate that significant differences exist between the 3 groups as well ($F = 3.23$, $p = 0.05$) with the mean score for Group C being significantly higher than Groups A & B.

To determine whether significant differences exists between the 3 groups of rabbits according to the onset of flaccid paralysis and sensory loss after aortic occlusion, as well as the initial recovery period from flaccid paralysis after reperfusion, a one way analysis of variance was used followed by Takey's test for multiple comparisons. The results indicate that significant differences exists between the 3 groups according to the onset of sensory loss after aortic occlusion ($F = 7.01$, $p = .0035$) with a mean for Group C being significantly higher than that of Group A. There are no significant difference between the 3 groups with respect to the onset of flaccid paralysis after occlusion and the onset of initial recovery after reperfusion.

To determine whether or not the onset of flaccid paralysis or sensory loss after aortic occlusion, and recovery period after aortic reperfusion are significant predictors of the final neurologic outcome, a logistic regression analysis was applied. The results indicate that of the 3 parameters, only the onset of flaccid paralysis is a significant predictor of the final neurologic outcome. (Wald's $x = 6.98$, $p = .0082$). Higher values

for the onset of paraplegia was observed to be correlated with higher scores for final neurologic outcome.

Histologic studies revealed varying degree of neuron degeneration manifested as dark staining bodies containing ill-defined nuclei and some manifesting disintegrating neuron bodies engulfed by macrophage and swollen axons with clean halos which are more clearly demonstrated in the anterior horns of all cases with neurologic grades of 0-2, and 60% (3/5) of those having Grade 3 .

DISCUSSION

Neurologic complications of aortic occlusion is an old topic of investigation and discussion . First mention of this phenomenon was made in 1667 by Stenosis and Swammerdam where experimental occlusion of abdominal aorta resulted in paraplegia of a rabbit (5). Hara and Lipin were apparently the first to report a case of spinal cord injury following resection of an abdominal infra-renal aortic aneurysm which was manifested by urinary retention and fecal incontinence (6). Since then, many studies have been made and devoted to the prevention of this serious neurologic complication . Various methods were used like heparinized shunts/ creation of bypasses, injections distal to the occlusion to increase oxygen level and pressure, or lowering the metabolism of nervous tissue by hypothermia. Unfortunately, despite all these known interventions, the incidence of

permanent ischemic spinal cord injury especially following thoracoabdominal aortic aneurysmectomy remains high.

Since the complication of paraplegia is related to permanent of transient ischemia of the spinal cord, oxygen free-radicals have been implicated as mediators of ischemia-reperfusion injury (IRI) not only in the cerebrospinal but also in various organ systems. This is shown in studies demonstrating that these radicals are abundantly produced in ischemic tissues accounting for a substantially large part of the damage that results.

What exactly is a free radical? A free radical is simply a molecule containing an odd number of electrons, and thus, considered to contain an open bond or a half bond, rendering it chemically active. This characteristic enables free radicals to participate in chain reactions, which may be thousands of events long. e.g. peroxidation of unsaturated fatty acids by molecular oxygen.

McCord et. al presented evidences that the xanthine oxidase system is the primary source of oxygen derived free radicals in the setting of ischemia followed by reperfusion. Initial products are the superoxide anion and hydrogen peroxide. These compounds may combine in a trace metal-catalyzed reaction to form hydroxyl radical, which is more reactive than either of its precursor.

Among the active oxygens. hydroxyl radicals are the most reactive which damage proteins, break deoxyribonucleic acids (DNA) and promote lipid peroxidation. They may also arise from ionizing radiation, ultrasound, lithotripsy, lyophilization, ozone and

ethanol metabolism , iron solutions and brain guanidino compounds. Antioxidants that quench active oxygens are well documented. but inability of such popular agents such as catalase and superoxide dismutase to cross the blood brain barrier limits their actions as protectors of the central nervous system.

Dimethylthiourea, also a hydroxyl radical scavenger was demonstrated to prevent paraplegia following aortic cross- clamping in rabbits (1). However, it has yet to undergo clinical trials. Another factor is it's unavailability in the market.

Bio-catalyzer a p No. 11 (Bio-normalyzer), on the other hand, is a white, sweet, granular, natural health food commercially sold in Japan and the Philippines. It is made by yeast fermentation of Carica Papaya Linn.(a widely known Philippine herb), Pennisetum pupureum Schum. (Napier grass), Sechium edule Swartz (vegetable) and glucose as the main carbon source.

Reports by L.A. Santiago et.al. have shown that Bio-normalyzer (BN) potently scavenged hydroxyl radicals, quenched superoxide and 1,1 diphenyl-2picrylhydrazyl (DPPH) radicals, as well as inhibited the thiobarbiturate acid reactive substances (TBARS) in FeCl3 induced epileptic focus in rats (4). In another study, they have demonstrated that BN can also significantly reduce membrane lipid peroxidation in cerebral ischemia reperfusion injury (IRI) thereby affording cerebroprotection during transient CNS ischemia.

Our study revealed a significant difference in the final neurologic outcome among the 3 groups with the treatment group (C) having the highest mean neurologic grading of

2.4 vs. 1.3 and 1.4 in Groups A & B, respectively. Although a significant neurologic improvement was noted in Group C compared to the other 2 groups, the overall paraplegia rate (Gr 0,1) was still relatively high at 30%, which is low compared to that reported by Wissenlink, et. al. (1), who had complete neurologic recovery in all 10 animals after treatment with dimethylthiourea (DMTU). It's possible though ,that the optimum dose, duration , as well as the route of administration are yet to be determined and these may influence and improve further the final neurologic outcome in future studies.

The onset of paraplegia after temporary aortic occlusion in secs. was also shown to have a significant relationship to the final neurologic outcome and may likewise serve as a predictor of neurologic recovery. The relationship is directly proportional, which is, the longer the onset of paraplegia after aortic occlusion, the better is the chance of attaining neurologic recovery.

The etiology of ischemic spinal cord injury is a multifactorial phenomenon. Considerations must be made regarding the anatomic, physiologic and biochemical aspects of any surgery that would involve temporary occlusion of the aorta. The basic denominator however, is the presence of ischemia, followed by reperfusion , producing oxygen-derived free radicals responsible for the cellular damages. The phenomena of ischemia reperfusion injury is demonstrated vividly in different studies in the past. The fact that administration of a radical scavenger in the form of Bio-normalyzer significantly

improved the neurologic outcome of the tested animals is an indirect evidence of the role that oxygen- derived radicals play in ischemia-reperfusion injury of the spinal cord.

CONCLUSION

Our results support the fact that hydroxyl radicals may play an important role in ischemia-reperfusion injury of the spinal cord. Treatment with Bio-catalyzer No. 11 (Bio-normalyzer) can improve the neurologic outcome, if not prevent paraplegia following aortic cross clamping in rabbits. The onset of paraplegia after aortic occlusion may also serve as a predictor for the final neurologic outcome after temporary aortic occlusion in experimental animals.

TABLE 1
FINAL NEUROLOGIC OUTCOME
(GRADE)

	A (Control)	B (Placebo)	C (Bio-normalyzer)
1	1	1	3
2	0	1	1
3	1	1	1
4	1	3	3
5	1	1	2
6	2	1	4
7	0	1	4
8	4	3	2
9	2	1	3
10	1	1	1

TABLE 2
ONSET OF FLACCID PARALYSIS AFTER
AORTIC OCCLUSION
(SECONDS)

	A	B	C
1	60	100	80
2	80	60	60
3	40	30	40
4	30	50	80
5	60	50	40
6	90	90	100
7	30	90	90
8	90	100	50
9	80	30	40
10	40	50	60

TABLE 3
ONSET OF SENSORY LOSS AFTER AORTIC OCCLUSION
(SECONDS)

	A	B	C
1	160	300	200
2	200	240	180
3	160	400	240
4	180	360	600
5	160	200	400
6	190	120	180
7	60	300	300
8	120	120	240
9	90	200	480
10	60	240	240

TABLE 4
INITIAL RECOVERY FROM FLACCID PARALYSIS
AFTER REPERFUSION (MINUTES)

	A	B	C
1	120	300	120
2	-	-	180
3	180	-	100
4	240	240	80
5	-	240	120
6	240	120	70
7	-	-	240
8	120	100	180
9	90	240	180
10	240	180	240