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Philip J. Migliore, M.D.
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Dear Dr. Migliore:

Enclosed please find the annual report to the Moran Foundation regarding the project "Clinical Application of Rapid Amino Acid Analysis" (1-85-0013) (01-85-0013). I believe that we have made significant progress in our research, but as always those insights we've gained have led inevitably to more questions. Given the lateness in the year that the funds became available to us, and our current analytical lull due to maternity leave by our technician; we respectfully request that the unexpended funds for our project be carried over to 1987.

Thank you. As always, should you are members of the Scientific Review Committee have questions about the project, please don't hesitate to contact me.

Sincerely,

J. Clay Goodman, M.D.

MORAN FOUNDATION
PROJECT ANNUAL REPORT
DECEMBER 1986

CLINICAL APPLICATION OF RAPID AMINO ACID ANALYSIS
(1-85-0013)

J. Clay Goodman
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Clinical Application of Rapid Amino Acid Analysis
Goodman/Ou/Robertson

Introduction

Over the past two years we have used Moran Foundation funds to measure whole blood concentrations of amino acids in head injuried patients using high pressure liquid chromatography with post-column ninhydrin derivatization. The measurements have been performed serially in the days following injury, and this information has been linked to cerebral metabolic, systemic metabolic, and clinical course data which is also obtained on these patients during their hospitalization. Amino acid measurements were done on arterial blood and internal jugular blood so that arteriovenous differences in concentrations across the cerebral circuit were obtained. The AV differences when coupled with simultaneous measurement of cerebral blood flow using nitrous oxide as the diffusable indicator in a Kety-Schmidt paradigm, permitted computation of cerebral metabolic utilization rates for the individual amino acids. In addition to the amino acids, we measured cerebral metabolic rates for oxygen, glucose, and lactate. This study provides unique information about cerebral metabolism following head injury and is laying the foundation for design of rational nutritional and pharmacological intervention in this condition.

Background

The brain is critically dependent on systemic sources for all of the substrates necessary to sustain its metabolic activities. Normally, the

brain oxidizes glucose almost exclusively for energy production at a rate of 110-145 grams/day which accounts for about 25% of resting systemic glucose consumption. The brain also normally has a net influx of amino acids averaging 50 μmoles/minute, or roughly 25% of the uptake of amino acids by the splanchnic tissues in the postabsorption state.

In the past, the metabolic state of the brain in the post-injury state was thought to be similar to that seen in fasting since most injuried patients did not receive significant nutritional support immediately following injury. In the fasting state, there is a systemic reduction in circulating glucose and amino acids accompanied by fat mobilization with release of ketone bodies. The brain adapts to these alterations by increasing utilization of ketone bodies such that by three weeks of fasting fully 60% of the cerebral energy demands are met by oxidation of acetoacetate and beta-hydroxybutyrate. Despite reductions in circulating amino acids, cerebral demand for these substrates remains constant.

The systemic metabolic environment following head injury is not, however, really the same as that seen in fasting, and an entirely different pattern of blood concentrations of glucose, lactate, pyruvate, ketones, and amino acids have been described. Our study was designed to examine systemic and cerebral metabolic changes in the post-injury state.

Methods

A total of 27 head injuried patients were examined daily for the first 7 days following injury. None of these patients had underlying medical conditions or any significant injury other than their neurological injury,

and none were septic during the study period. All of the patients were managed on a protocol which emphasized early evacuation of intracranial hematomas, monitoring of intracranial pressure with aggressive management of increased pressure, controlled ventilation, and avoidance of secondary injury to the brain. Nineteen of the patients had a surgical procedure in which hematoma and nonvital brain tissue was removed whereas the remaining eight patients required only placement of a ventricular catheter for pressure monitoring. Routine medications included phenytoin, morphine, and antibiotics. Intracranial pressure was controlled using hyperventilation and mannitol, and in three instances barbiturate coma was employed. All patients received D5-1/2NS as their only caloric source (400 kcal/day) for the first 3 days after admission followed by enteric or parenteral feeding as bowel function permitted. Twenty of the twenty-seven patients received corticosteroids.

Control arterial amino acid concentrations were obtained in 11 neurosurgical patients undergoing elective craniotomy on the day of operation and on the first post-operative day. Additionally, arterial amino acids were measured in 8 patients undergoing non-neurosurgical operations. In addition, the results of our studies were compared with entirely normal controls published in the literature using similar analytical methods.

Cerebral blood flow was measured by the Kety-Schmidt technique using nitrous oxide as the diffusible indicator. For this purpose, an 18 gauge Teflon catheter was inserted in the internal jugular vein and

positioned so that the tip was in the jugular bulb. The catheter was placed on the side of the most severe injury, or on the right when the injury was diffuse. Arterial samples were obtained from standard radial artery catheters. Ten percent nitrous oxide was introduced into the inspired gases in a stepwise fashion, and ten timed 500µl samples of arterial and venous blood were collected during the first 15 minutes of nitrous saturation and nitrous oxide concentrations were measured using an infrared nitrous oxide analyzer. Cerebral blood flow (CBF) was calculated using the Kety-Schmidt equations. The coefficient of variation of repeated CBF measurements was 3%.

Simultaneously with the CBF measurements, arterial and venous blood samples were obtained for measurement of blood gases, oxygen saturation, hemoglobin, lactate, glucose, and amino acid concentrations; and arterial blood was obtained for measurement of catecholamine concentrations. Cardiac output was measured by thermodilution at the time the CBF was obtained. Daily urinary nitrogen loss was measured in 24 hour urine samples by the chemiluminescence technique.

Results

Summarizing a large amount of information:

1. The head injuried patients were hypermetabolic systemically as indicated by elevated systemic oxygen consumption, elevated cardiac index, and elevated urinary nitrogen excretion.

2. Arterial catecholamine levels were elevated throughout the course.

3. Head-injuried patients were hyperglycemic throughout with

maximal levels being seen on the day of injury. Lactate was also elevated.

4. Arterial amino acids followed three patterns of evolution in the week following injury:

Alanine, glutamic acid, and taurine were elevated from the day of injury, and except for glutamic acid gradually decreased toward normal over the days following injury.

Lysine, phenylalanine, tyrosine, and methionine were normal or elevated on the day of injury, decreased initially, and then increased significantly during post-injury days 3-6.

Valine, leucine, isoleucine, threonine, serine, arginine, aspartic acid, and glycine were decreased on the day of injury and immediate post-injury days and then returned toward normal.

The net cerebral flux of amino acids followed the same general pattern of evolution over time as did the arterial concentrations of the amino acids. On days when the availability of the individual amino acid was increased as reflected in elevation of arterial concentration; the net flux tended to be positive. When the availability was decreased; flux was close to zero or there was net efflux of the amino acid. The three amino acids that were increased on the day of injury, taurine, alanine, and glutamic acid, had a net cerebral influx; especially on day 1 when the arterial levels were highest. The amino acids lysine, methionine, phenylalanine, and tyrosine had a net cerebral influx on day 1, and a net efflux on day 2 as their arterial levels initially decreased, followed by restoration of influx as the arterial levels increased once again. The

amino acids that were initially decreased in arterial blood displayed net efflux or a net exchange close to zero.

There was no relationship between the amino acid flux and severity of injury, cerebral blood flow, cerebral oxygen consumption, or cerebral lactate production. In fact, the cerebral amino acid flux related only passively to arterial amino acid concentration as described above. This situation contrasts sharply with the avid continuous uptake of amino acids over a large range of arterial concentrations by the brain under normal and fasting conditions

5. Cerebral metabolic utilization of glucose and oxygen were consistently low throughout the study period; and there was no relationship between glucose and oxygen arterial availability and cerebral utilization. Cerebral lactate production increased implying greater reliance on anaerobic metabolism for energy production.

Summary

Following head injury, there is systemic hypermetabolism which differs from the metabolic response seen in simple fasting. Accompanying the increase in systemic metabolism is a decrease in cerebral utilization of glucose and oxygen with an increase in lactate production. Following injury, the brain's normally active positive uptake of all amino acids is abolished and is replaced by passive influx or efflux as a function of arterial amino acid concentration. It is possible that as clinical recovery takes place, normal active uptake of amino acids will ensue. Until then,

however, it might be possible and desirable to alter systemic amino acid concentrations by nutritional supplementation or pharmacological manuvers so that net cerebral amino acid influx obtains. The effects of such therapeutic interventions must be studied in terms of impact on cerebral oxygen metabolism, glucose utilization, lactate production, and clinical outcome. Continued study of the basic alterations in cerebral and systemic metabolism as well as the impact of therapy on these forms the basis of our request that funding for this work continue into 1987.

Budget

According to our records, we still have $3017.55 of the original $5300.00 authorized for this project. The incomplete expediture of these funds reflects several factors including longer column life than expected obviating replacement, interruption of analytical activity due first to the movement of the HPLC equipment to the TCH laboratory at 8080 Stadium and then due to maternity leave by our technician Susan Bejot, and authorization of the funds by the Scientific Advisory Committee relatively late in the year (June 5, 1986) due to appropriate concerns by members regarding the original application. As indicated above, we respectfully request that the remaining funds remain available to us for use in 1987 for column and reagent costs. We anticipate resumption of analytical activity in February 1987 when Ms. Bejot returns, and we are continuing to collect samples in her absence. Additionally, I am exploring the possibility of the amino acid analysis being performed in our lab at Baylor

using less expensive columns and reagents; specifically, using precolumn dansyl derivatization with separation on a C18 column.

Publications & Presentations

This work has resulted in acceptance of two abstracts for presentation at national meetings:

1. "AMINO ACID ALTERATIONS FOLLOWING HEAD INJURY", J.C. Goodman, Ching-Nan Ou, C.S. Robertson, and S. Bejot. Presented at the joint meeting of the American Association for Clinical Chemistry & Canadian Society of Clinical Chemists, July 13-18, 1986. Chicago, Illinois. The poster was visited by several workers in the area of critical care nutrition, and the comments made were helpful and favorable. The poster was also commented upon in the October 1986 issue of the trade magazine Laboratory Management in its coverage of the convention.

2. "ALTERATIONS IN CEREBRAL AVAILABILITY OF METABOLIC SUBSTRATES AFTER HEAD TRAUMA", C.S. Robertson, J.C. Goodman, CN Ou, and S. Bejot. Accepted for presentation at the 11th Clinical Congress of the American Society for Parenteral and Enteral Nutrition (ASPEN), in January 1987, New Orleans, La.

A manuscript detailing the amino acid work in the context of other alterations in systemic and cerebral metabolism is in preparation, and is almost complete for submission to the Journal of Neurosurgery.

Grants & Contracts

The demonstrated close scientific cooperation which has occurred between members of the Departments of Pathology and Neurosurgery in the conduct of this project, and the willingness of both departments to provide seed money for these investigations places us in a good position to compete for funds from the federal government as well as private sources.

Recently, Baylor has been named one of four penetrating head injury centers across the country by the U.S. Army; this designation provides support for data processing, nursing and technical staff, and design and implementation of clinical trials in penetrating head injury. While many factors went into the decision to designate Baylor one of these centers, the novel and unique investigations in cerebral and systemic metabolism supported in part by the Moran Foundation no doubt gave credibility to our position as center on the forefront of head injury research.

We are currently planning major NIH grant applications in 1987 to extend and render fiscally autonomous our present investigations, but given the long gestation periods of such applications and the current uncertainty of federal funding, continued support by the Moran Foundation would be most welcome.