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Short communication

Increasing glucose load while maintaining normoglycemia does not evoke neuronal damage in prolonged critically ill rabbits

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SUMMARY

Background & aims: Preventing severe hyperglycemia with insulin reduced the neuropathological alterations in frontal cortex during critical illness. We investigated the impact of increasing glucose load under normoglycemia on neurons and glial cells.
Methods: Hyperinflammatory critically ill rabbits were randomized to fasting or combined parenteral nutrition containing progressively increasing amounts of glucose (low, intermediate, high) within the physiological range but with a similar amount of amino acids and lipids. In all groups, normoglycemia was maintained with insulin. On day 7, we studied the neuropathological alterations in frontal cortex neurons, astrocytes and microglia, and MnSOD as marker of oxidative stress.
Results: The percentage of damaged neurons was comparable among all critically ill and healthy rabbits. Critical illness induced an overall 1.8-fold increase in astrocyte density and activation status, largely irrespective of the nutritional intake. The percentage of microglia activation in critically ill rabbits was comparable with that in healthy rabbits, irrespective of glucose load. Likewise, MnSOD expression was comparable in critically ill and healthy rabbits without any clear impact of the nutritional interventions.
Conclusions: During prolonged critical illness, increasing intravenous glucose infusion while strictly maintaining normoglycemia appeared safe for neuronal integrity and did not substantially affect glial cells in frontal cortex.

1. Introduction

Persistent hyperglycemia is a common complication of critical illness that may contribute to acute brain injury and dysfunction and has been associated with long-term adverse cognitive sequelae in survivors.[1,2] Maintaining blood glucose levels normal with insulin infusion was suggested to be neuroprotective. Indeed, studies in human and animal brain specimens showed that strict blood glucose control during critical illness attenuated neuropathological alterations at the level of neurons, astrocytes and microglia in vulnerable areas of the brain.[2] We also demonstrated clinical benefit, as maintenance of normoglycemia lowered intracranial pressure levels and the risk of seizures in patients with isolated brain injury and reduced the incidence of neuromuscular complications of critically ill patients admitted to the surgical or medical intensive care unit.[3] However, maintaining normoglycemia with insulin remains a controversial intervention. Indeed, several other mostly small studies failed to reproduce these clinical benefits while uniformly observing an increased risk of severe hypoglycemia, though methodological quality may be an important issue.[4] Importantly, long-term follow-up of critically ill children randomized to strict glycemic control versus usual care showed that this intervention did not increase the incidence of poor outcomes and did not compromise, but rather improved, neurocognitive development four years after ICU admission.[5]

In theory, glucose toxicity during critical illness could have a dual origin. Indeed, high blood glucose levels per se may be detrimental, but also the amount of glucose intake may play a role. As such, high intake of glucose could theoretically overload the brain cells. Supplementation of enteral nutrition with parenteral

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; GLUT-4, glucose transporter-4; IV, intravenous; MnSOD, manganese superoxide dismutase.

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nutrition to achieve the caloric target has been recommended to counteract the hypercatabolic state of critical illness. Interestingly, however, early initiation of supplemental parenteral nutrition when enteral nutrition is insufficient has recently been shown to delay recovery of critically ill patients in our setting.[6] The impact of this intervention, while maintaining normoglycemia, on the central nervous system is so far not known.

In the present experimental neuropathological study, we addressed the impact of glucose intake on neurons and glial cells during critical illness. Therefore, we varied the intravenous glucose load under normoglycemia in a rabbit model of sustained hyperinflammatory critical illness.

2. Materials and methods

2.1. Study design

The KU Leuven Ethical Review Board for Animal Research approved the study (Protocol P05110). Animals were treated according to the Principles of Laboratory Animal Care (US National Society for Medical Research) and the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

Our catheterized, fluid-resuscitated third-degree burn injury rabbit model of prolonged critical illness has been extensively described previously.[7,8] Male New Zealand white rabbits were instrumented under general anesthesia induced with an intramuscular injection of 30 mg/kg ketamine and 0.15 ml/kg medetomidine and maintained by 1.5% isoflurane inhalation. A catheter for repeated blood sampling was placed in the left carotid artery and another one for continuous intravenous nutrition and insulin administration was placed in the left jugular vein. After performing a paravertebral block with 5 ml 1% Xylocaine (Astra-Pharmaceuticals, Brussels, Belgium), a full-thickness third-degree burn wound of 15–20% total body surface area was inflicted on the flanks (painless because cutaneous nerve ends are destroyed), which was covered dry and clean by a home-made jacket. Continuous fluid resuscitation was started with Hartmann solution (Baxter, Lessiness, Belgium) supplemented with 5% glucose at 16 ml/h. Glycemia was controlled to normal fasting levels (80–110 mg/dl, 4.4–6.1 mmol/l) under continuous intravenous insulin infusion (Actrapid, Novo Nordisk, Begsvaerd, Denmark). In the evening, a subcutaneous 0.2 mg/kg piritramide injection (Dipidolor, Janssen-Cilag, Beerse, Belgium) was given to prevent post-operative pain from surgery and inflammation around the burn injury. The critically ill rabbits were randomly allocated by sealed envelopes to four groups to receive different feeding regimens from the next morning onwards. The animals were deprived from oral feeding, but received parenteral nutrition according to randomization to assure uptake of the exact amount of administered nutrients and had free access to water and a small amount of hay. A fasted group received only minimal glucose to prevent/treat hypoglycemia (Table 1). The other three groups received parenteral nutrition based on Clinomel N7 (Baxter; Clinitec, Maurepas Cedex, France), containing a same amount of amino acids and lipids but with a low, intermediate or high amount of glucose, infused at 10 ml/h. In all groups, blood glucose control to 80–110 mg/dl was continued until the end of the study. On day 7, animals were anesthetized and sacrificed. Brain tissue samples were harvested at the level of frontal cortex and were embedded in paraffin or stored at −80°C until analysis. The study was continued until in each group at least ten rabbits survived until day 7. For establishment of healthy reference ranges, data and brain biopsies were collected from healthy rabbits.

2.2. Histology

Neuropathological studies were performed as previously described.[2] The percentage of damaged neurons (shrunken eosinophilic cytoplasm, pyknotic nuclei after hematoxylin-eosin staining) was determined by counting of the cells with ImageJ 1.36b® software (Wayne Rasband, NIH, Bethesda, MD). Reactive astrocytes were stained for Glial Fibrillary Acidic Protein (mouse monoclonal anti-GFAP, 1/400, Millipore, Brussels, Belgium). Astrocytes were considered activated when an increase in the size of GFAP-positive cells was observed and when they presented with longer, thicker processes. Density of GFAP-positive astrocytes and

Table 1
Nutritional intake and metabolic control of critically ill rabbits.

Group	Fasted ($n = 11$)	Low-glucose-load ($n = 12$)[a]	Intermediate-glucose-load ($n = 10$)	High-glucose-load ($n = 11$)	*P*-value
Parenteral nutrition					
IV Glucose, kcal/day	14 (1)	178 (13)	235 (6)	301 (15)	<0.0001
IV Amino acids, kcal/day	0	14 (1)	14 (1)	14 (1)	<0.0001
IV Lipid, kcal/day	0	31 (2)	31 (1)	31 (2)	<0.0001
IV Total, kcal/day	14 (1)	223 (16)	280 (7)	346 (17)	<0.0001
Blood glucose levels					
Baseline, mg/dl	157 (18)	155 (21)	152 (21)	172 (33)	0.40
Mean of all blood glucose measurements, day 1–7, mg/dl	91 (16)	104 (9)	103 (6)	105 (7)	0.003
SD of all blood glucose measurements, mg/dl	68 (5)	74 (7)	74 (4)	75 (6)	0.048
Insulin					
Dose administered, IU/day	1.8 (0.6)	6.3 (1.2)	12.5 (5.6)	13.4 (6.1)	<0.0001
Plasma levels, mIU/l	20 (13)	56 (15)	122 (116)	181 (188)	<0.0001
Hypoglycemic events (≤40 mg/dl)					
n/total	4/11	2/12	0/10	1/11	0.13
n of episodes per animal	1–2	1	0	2	
Other metabolic parameters					
pH	7.38 (0.04)	7.39 (0.04)	7.39 (0.05)	7.39 (0.05)	0.91
$PaCO_2$, mmHg	28 (5)	30 (7)	36 (3)	26 (7)	0.005
PaO_2, mmHg	99 (17)	103 (13)	106 (30)	99 (11)	0.65
Hemoglobin, g/dl	10 (1)	10 (1)	10 (1)	11 (1)	0.47
Lactate, mmol/l	0.5 (0.4)	1.3 (1.0)	0.9 (0.3)	1.0 (0.6)	0.02

Data are shown as mean (SD) unless indicated otherwise. The four critically ill groups were compared by Kruskal–Wallis test.
[a] In the low-glucose-load group, 13 surviving rabbits had been included, but for one of these rabbits no usable sample was available. IV: intravenous.