Oral Sucrose for Heel Lance Increases Adenosine Triphosphate Use and Oxidative Stress in Preterm Neonates

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Objective To examine the effects of sucrose on pain and biochemical markers of adenosine triphosphate (ATP) degradation and oxidative stress in preterm neonates experiencing a clinically required heel lance.

Study design Preterm neonates that met study criteria (n = 131) were randomized into 3 groups: (1) control; (2) heel lance treated with placebo and non-nutritive sucking; and (3) heel lance treated with sucrose and non-nutritive sucking. Plasma markers of ATP degradation (hypoxanthine, xanthine, and uric acid) and oxidative stress (allantoin) were measured before and after the heel lance. Pain was measured with the Premature Infant Pain Profile. Data were analyzed by the use of repeated-measures ANOVA and Spearman rho.

Results We found significant increases in plasma hypoxanthine and uric acid over time in neonates who received sucrose. We also found a significant negative correlation between pain scores and plasma allantoin concentration in a subgroup of neonates who received sucrose.

Conclusion A single dose of oral sucrose, given before heel lance, significantly increased ATP use and oxidative stress in premature neonates. Because neonates are given multiple doses of sucrose per day, randomized trials are needed to examine the effects of repeated sucrose administration on ATP degradation, oxidative stress, and cell injury. *(J Pediatr 2013;163:29-35)*.

Premature neonates experience many painful procedures as part of their standard care in the neonatal intensive care unit.[1,2] To prevent or treat procedural pain, the use of oral sucrose is recommended by many national and international clinical guidelines on the basis of results from multiple randomized clinical trials in which investigators determined sucrose to be effective in reducing signs of pain.[3] However, there are no studies to date in which investigators examine the effects of sucrose, a disaccharide of fructose and glucose, on neonatal cellular adenosine triphosphate (ATP) metabolism, despite the well-documented relationship between fructose metabolism and reductions in ATP synthesis in adult animals,[4] in children ages 11 months to 12 years,[5] and in healthy adults.[5,6]

Inhibition of ATP synthesis, as a consequence of sucrose administration, may reduce a premature neonate's already modest ATP stores.[7] In addition, evidence shows that the administration of sucrose does not attenuate the tachycardia that often accompanies painful procedures.[8] In neonatal pigs and newborn lambs, tachycardia significantly increased glucose oxidation and myocardial oxygen requirement,[9] which paralleled significant reductions in phosphocreatine/ATP and significant elevations in adenosine diphosphate (ADP) and inorganic phosphate.[10] More importantly, sucrose may not be an effective analgesic as evidenced by its lack of impact on neonatal nociceptive circuits in the brain and spinal cord.[11] Together, these considerations imply that oral sucrose administration may alter ATP metabolism and may have adverse cellular effects in neonates with limited energy stores.

The aim of this study is to examine the effects of a single dose of oral sucrose on behavioral/physiological markers of pain and biochemical markers of ATP metabolism and oxidative stress in premature neonates experiencing a clinically required heel lance. The heel lance was chosen because it is a frequent painful procedure in the neonatal intensive care unit, as shown in 26 different clinical trials.[12] Pain was quantified using the Premature Infant Pain Profile (PIPP),[13] ATP metabolism was quantified by measuring plasma concentrations of purines (hypoxanthine, xanthine, and uric acid), which are well-documented markers of ATP use and breakdown, and oxidative stress, measured as plasma concentrations of allantoin, a well-accepted in vivo free radical marker.[14]

Methods

We conducted a prospective double-blind randomized controlled study at Loma Linda University Children's Hospital neonatal intensive care unit. Study

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
GLUT	Glucose transporter
PIPP	Premature Infant Pain Profile
NNS	Non-nutritive sucking

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This study is funded by National Institutes of Health (RO1 NR011209). The authors declare no conflicts of interest.

 http://dx.doi.org/10.1016/j.jpeds.2012.12.088

protocol and informed consent documents were approved by the Loma Linda University Children's Hospital Institutional Review Board. Subjects included in the study were premature infants ≤36.5 weeks' gestation who: (1) weighed ≥800 g; (2) had a central catheter in place; and (3) required a heel lance. Exclusion criteria included neonates: (1) with unstable oxygenation and hemodynamic status; (2) receiving opioids or sedatives or any antiepileptic medications; (3) diagnosed with intraventricular hemorrhage ≥ grade 3; or (4) with facial or multiple congenital anomalies that might alter the pain response. The heel lance was performed for an accurate measurement of blood glucose from neonates receiving glucose-rich total parenteral nutrition through a central catheter. Parents of premature infants who met study criteria were approached for informed consent as soon after birth as possible. With consent, subjects were randomized into 1 of 3 groups: (1) control; (2) placebo with non-nutritive sucking (NNS, or pacifier); or (3) sucrose (Sweet-Ease; Children's Medical Ventures, Phillips Healthcare, Andover, Massachusetts) with NNS (**Figure 1**; available at www.jpeds.com). Randomization was performed by a research pharmacist, who used a permuted block randomization table generated by the study statistician.

The experimental procedure is described in **Figure 2** (available at www.jpeds.com). Investigators collaborated with the clinical staff to obtain a sample of approximately 0.8 mL of blood from a central catheter before ("0" minute) and 5 minutes after the heel lance to measure purine and allantoin levels. In control neonates who did not receive a study drug or undergo a heel lance, similar samples were collected at "0" and 5 minutes from baseline. The time period of 5 minutes after heel lance for blood sample collection was based on previous investigations, which showed plasma levels of purines and organic hydroperoxides significantly increasing 5 minutes after conditions such as incomplete ischemia.[15] These data were validated by unpublished preliminary studies in our laboratory, where we found increases in plasma purines compared with baseline five minutes after heel lance, and purine values that were less than baseline, 20-30 minutes after heel lance. Blood samples were centrifuged within 5 minutes to separate the plasma which was then stored at −80°C. All samples were analyzed within 1 week of acquisition.

Heel Lance Procedure and Administration of Study Drug

The study drug was prepared immediately before the experimental procedure by the research pharmacist and labeled as "study drug" to ensure blinding. The dose of sucrose was based on previously published studies in premature infants.[12,16-18] Neonates randomized to the sucrose group received a single dose of 24% sucrose in the following volumes: 2 mL for neonates >2 kg, 1.5 mL for neonates 1.5-2 kg, and 0.5 mL for neonates that were <1.5 kg. The study drug was administered slowly via syringe to the anterior tongue along with a pacifier (NNS) 2 minutes before the heel lance. Multiple studies showed that sucrose was most effective when given approximately two minutes before heel lance.[8,18-23] Neonates randomized to the placebo group received an equal volume of sterile water to the anterior portion of the tongue along with a pacifier. The neonate's face was videotaped by trained research staff to record facial action at "0" minutes, during the heel lance and up to 30 seconds post heel lance.

Pain Assessment

To assess pain, we used the PIPP, an instrument designed to assess acute pain in preterm neonates.[13] This scoring system includes seven items, each graded from 0 to 3. Two items describe baseline characteristics of the neonate (gestational age and behavioral state), 2 items are derived from physiologic measurements (heart rate and oxygen saturation), and 3 items describe facial actions (brow bulge, eye squeeze, and nasolabial furrow). Baseline pain was scored before the heel lance (0 minute) during a 30-second window. Procedural pain was scored from the time of heel lance to 30 seconds after the lance. Facial actions were recorded with a digital camera with real-time counter that allowed for intensive slow motion stop frame, videocoding, and playback. Previous work on validation of the PIPP score showed an ability to differentiate painful from non-painful or baseline events.[13,24]

Measurement of Purines

Purine metabolites were measured as previously published by our laboratory.[25] Specifically, plasma was removed, transferred to separate Eppendorf tubes, and immediately centrifuged in Eppendorf 5702R (Pittsburgh, Pennsylvania) centrifuge, for 30 minutes at 18 000 g. The supernatant was transferred to Microcon centrifugal filter devices (Millipore Corp, Bedford, Massachusetts), 200 μL per device, and spun for 90 minutes at 14 000 g, 4°C. Filtrate was removed, and 150 μL was transferred to an Eppendorf tube containing 1×10^{-7} mol of 2-aminopurine (internal standard). High-performance liquid chromatography (Waters 996 PDA, 715 Ultra Wisp Sample Processor; Millipore Corp) analysis was done in the same day, or the tubes were frozen at −80°C until analysis. Previous analysis via high-performance liquid chromatography of plasma demonstrated that purines remained stable with freezing.

Three 45-μL injections were used for each sample onto a Supelcosil LC-18-S 15 cm × 4.6 mm, 5-μm column (SGE, Austin, Texas), with the following isocratic conditions: 50 mM ammonium formate buffer, pH 5.5, flow rate 1.0 mL/min. Hypoxanthine, xanthine, and uric acid were quantitated by obtaining peak areas at appropriate retention and wavelengths.[26] Once the peak area of 2-aminopurine at approximately 10.8 minutes and 305 nm was determined, the area ratios of hypoxanthine, xanthine, and uric acid to 2-aminopurine were determined and converted to micromolar concentrations using standard curves. Samples were analyzed in triplicates and values with a coefficient of variation of less than 10% were included in the final analyses. The limits of