Age-related differences of intraischemic gap junction uncoupling in hearts during ischemia

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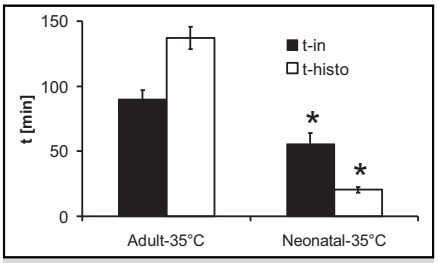
ABSTRACT

Objective: Myocardial ischemia leads to energetic, morphologic, metabolic, and functional alterations. To evaluate differences in ischemia tolerance between neonatal and adult hearts, we investigated gap junction uncoupling (GJU) and its correlation to myocardial intracellular edema formation during normothermic ischemia.

Methods: Hearts of landrace piglets (neonates, 7.4 ± 1.9 days of age, body weight 2.9 ± 0.5 kg, n = 5 and adults, 84 ± 9 days of age, body weight 30.5 ± 3.9 kg, n = 5) were investigated. After we harvested the hearts, the bioelectrical impedance spectra were measured continuously during normothermic global ischemia (35°C). Spectra of the dielectric permittivity, ε'(frequency), and conductivity, σ(frequency), were calculated from the impedance measurements, and GJU was identified in the sigmoidal time course of ε' (13 kHz). The extracellular volume was estimated by the ratio σ (100Hz)/σ (1MHz). Dielectric data were correlated with electron-microscopical images.

Results: Intraischemic GJU was observed in neonates after 54 ± 9 minutes of ischemia and thus significantly earlier than in adults (90 ± 7 minutes, $P < .05$). A more than 20% increase of intercalated water was found in tissue samples of neonates after 20 ± 2 minutes, in contrast to adults after 137 ± 8 minutes ($P < .05$).

Conclusions: Intraischemic formation of edema and earlier GJU indicate faster intraischemic changes in neonates compared with adults. Intraischemic GJU and determination of intracellular water shifts are an experimental approach to establish the period of life-threatening damage. Because both parameters are linked and occur significantly earlier in neonates, they distinctly demonstrate the lower ischemia tolerance of neonatal hearts as both events interact. (J Thorac Cardiovasc Surg 2016;152:729-36)



Time of inflexion (*t-in*) obtained from the intraischemic time course of the dielectric permittivity, ε', at 13 kHz compared with the time *t-histo* (ie, time concerning histologic alterations), when the intercalated water area found by digital image processing reached 20% during ischemia (mean ± standard error of the mean, *significant compared with the corresponding parameter in the adult heart group, $P \leq .05$).

Central Message

In neonatal hearts, significantly faster edema formation and earlier gap junction uncoupling during ischemia were measured, indicating greater sensitivity compared with adult hearts.

Perspective

With the purpose to correlate edema formation in congenital heart surgery to intraischemic gap junction uncoupling, we investigated adult and neonatal piglet hearts under standardized conditions of normothermic ischemia. To our knowledge, this is the first study of intraischemic gap junction regulation in neonatal hearts ever assessed by electrical impedance spectroscopy.

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Despite the immense progress achieved in the surgical treatment of congenital heart malformations within the last 20 years, extensively edematous hearts frequently are seen in congenital heart surgery. Surgical procedures cause prolonged ischemia and thus provoke the formation of

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Abbreviations and Acronyms

ATP = adenosine triphosphate
bw = body weight
DSC = delayed sternal closure
ε′ = permittivity
ECSI = extracellular space index
GJU = gap junction uncoupling
t-histo = time concerning histological alterations
t-in = time up to the point of inflexion, in ε′ (t)

edema,[1] sometimes resulting in delayed sternal closure (DSC). The incidence of DSC varies greatly and depends on the congenital heart malformation and the surgical procedure, for instance, Stage 1 palliation for left heart syndrome.[2,3] Statistically significant risk factors for DSC are pathologies of myocardial malformations, duration of cardiopulmonary bypass and cross clamp times, as well as the age of the neonates.[4,5] Undoubtedly, extended cardiopulmonary bypass time, in particular, carries the risk of total tissue water accumulation, but the significance of ischemia-induced myocardial intracellular edema formation must not be underestimated.

DSC is considered an effective therapeutic option for neonates/infants at risk for hemodynamic, respiratory, or hemostatic instability early after congenital heart surgery. Frequently, myocardial edema delays sternal closure. To reduce such extensive intraoperative edema, one needs to evaluate mechanisms that may be involved in this pathophysiologic process (eg, cardioplegic strategies, temperature management).

Under ischemic conditions, anaerobic glycolysis becomes the major pathway of energy support. Both lactate and hydrogen ions are produced as end products.[6] A balanced pH value is necessary to maintain cellular functions and indirectly stabilizes intra- and extracellular water distribution caused by ion homeostasis in the intra- and extracellular space. Intraischemic lactate production in neonates is remarkably greater than in adults, whereas intracellular proton buffer capacity is significantly lower.[7,8] Consequently, lower ischemia tolerance in neonates can be explained by lower buffering reserve and by more intensive accumulation of glycolytic metabolites, leading to an early water shift from the extra- to the intracellular space. The result of this pathophysiologic process is a progressive myocardial intracellular edema that occurs during ischemia and during the first hours postoperatively.

Aside from the formation of edema, long-lasting ischemia in myocardial tissue induces gap junction uncoupling (GJU) and damage to cell membranes.[9] Gap junctions can be uncoupled by several substances[10,11] and potential intraischemic triggers, such as a decrease in adenosine triphosphate (ATP) content, increase in intracellular calcium concentration, or cellular acidosis. Therefore, GJU depends on the progressive ischemia that occurs during transition from life to death.[12] Consequently, the formation of edema and GJU are important parameters that indicate heart tissue damage during ischemia.

The intent of our study was to investigate the lower ischemia tolerance of neonatal hearts as compared with adult hearts, reflected in the formation of edema and GJU. Therefore, we examined adult and neonatal piglet hearts under standardized conditions of normothermic ischemia and assessed the formation of edema by the analysis of histologic images and measured GJU by electrical impedance spectroscopy. To our knowledge, this is the first study of intraischemic gap junction regulation in neonatal hearts ever assessed by electrical impedance spectroscopy.

MATERIALS AND METHODS

Experimental Procedure

The experiments were carried out in landrace piglets that received humane care according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health pub. 86-23, revised 1985). The neonatal piglets (neonates) had a mean body weight (bw) of 2.9 ± 0.5 kg and were between 6 and 10 days of age (n = 5, 7.4 ± 1.9 days). The adult pigs (adults) had a mean weight of 30.5 ± 3.9 kg, and their median age was 3 months (n = 5, 84 ± 9 days). All pigs were intubated and ventilated after premedication with midazolam (0.5 mg/kg bw) and ketamine (15-20 mg/kg bw). Anesthesia was sustained with midazolam (0.5 mg/kg bw), fentanyl (0.001 mg/kg bw), pancuronium (0.1 mg/kg bw), and isoflurane. Fentanyl was used in our experiments because it is administered routinely for analgesia in small children. It attenuates myocardial injury caused by high-dose adrenaline[13] and is known to provoke preconditioning. Neonatal and adult hearts, however, both benefit from the positive effects of fentanyl, so that the comparison of both groups is less affected.

After median sternotomy, both caval veins were clamped as well as the aortic and pulmonary artery, resulting in global ischemia. Afterwards, the hearts were directly harvested for our ex vivo experimental setup and immediately stored in an incubator at a humidity of 30% (according to Murphy[14]) and constant temperature of 35°C (Video 1). Normothermia was deliberately chosen to induce high ischemic myocardial stress and to provoke early myocardial injury.

Measurement of GJU and Monitoring of Changes in the Extracellular Volume

Electrical impedance spectroscopy method was used for the measurement of intraischemic GJU and to estimate the extracellular volume. The impedance measurements were performed by the use of electrodes in constant contact with the heart tissue surface. A probe consisting of 4 parallel electrodes was placed on the epicardial layer of the left ventricle parallel to the anterior interventricular branch of the left coronary artery. The outer 2 electrodes injected current to the tissue and the inner 2 sensed the voltage. With a Solartron 1260 impedance analyzer (Solartron Analytical, Leicester, United Kingdom) controlled by ImpDAQ V1.03 iba e. V. software (ImpDAQ, Bad Heiligenstadt, Germany), the complex electrical