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Clinical paper

Influence of core body temperature on Tryptophan metabolism, kynurenines, and estimated IDO activity in critically ill patients receiving target temperature management following cardiac arrest☆

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ABSTRACT

Background/aims: Temperature control improves neurological prognosis in comatose cardiac arrest (CA) survivors. Previous reports demonstrate that most affected patients show signs of significant systemic inflammation. In an effort to better characterize potential temperature-related effects on key inflammatory pathways, we investigate the course of Tryptophan (Trp) levels, Tryptophan catabolites (including kynurenines) and indoleamine-2,3-dioxygenase (IDO)-activity in post CA patients.
Material/methods: In an observational blinded endpoint analysis, a total of $n = 270$ serial samples from 20 post CA patients (63.1 ± 16.6 yrs., 45% shockable rhythm, mean time to return of spontaneous circulation (ROSC) 26.6 ± 16.0 min) treated with target temperature management (TTM) were analyzed. Core body temperatures, course of Trp, Trp catabolites (incl. kynurenines), and estimated IDO-activity were followed up for a maximum of 7 days after ROSC. Patients were followed up until hospital discharge or death and functional outcome was recorded.
Results: Over the 7-day observational interval, marked changes in Trp serum levels and IDO-activity were noted. In general, Trp serum levels but not IDO-activity seemed to parallel with the course of core body temperature. In explorative analyses, a correlation of Trp (rho = 0.271 (95%-CI: 0.16–0.38, $p < 0.0001$) and IDO-activity (rho = −0.155, 95%-CI: −0.27 to −0.037, $p = 0.01$) with core body temperature was observed. Linear mixed effect models revealed a positive significant association of core body temperature with Trp serum levels (Likelihood ratio test $\chi^2 = 6.35$, $p = 0.012$). In patients with good (vs. unfavorable) outcome, a tendency toward higher Trp serum levels, lower IDO-activity, and lower Kynurenic acid levels was noted.
Conclusions: We observed significant changes in Trp catabolism and IDO-activity that appeared temperature associated in post CA patients. Under hypothermia, decreased serum levels of Trp and increased IDO-activity were noted. We speculate from our data that IDO-induction during hypothermia contributes to the previously described increased susceptibility to infection or sepsis under reduced temperatures.

Introduction

After initial successful cardiopulmonary resuscitation (CPR), the neurological outcome is of particular importance and predicting neurological outcomes remains a major clinical challenge on the ICU.[1–5] Key factors to determine neurological outcome after cardiac arrest (CA) include, among others, underlying disease characteristics, degree of CA-induced (cerebral) hypoxia and

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ischemia/reperfusion injury, and potential acquired organ failures. From a clinical perspective, however, it seems important to note that temperature control (e.g. via target temperature management [TTM]) in the early post-CA phase is associated with significant improvements in neurological outcomes.[6] However, temperature control does not per se prevent a significant "global" inflammatory response.[7–12] Interestingly, the magnitude of this "post-CPR systemic inflammatory response" may often compare to life-threatening severe invasive infection[13] and may also be observed in patients without severe aspiration. In addition, although mounting evidence speaks to the fact that temperature control is associated with significantly improved post CA outcomes, TTM-application was previously associated with increased infection rates.[14] However, the underlying mechanisms leading to increased susceptibility to infection under TTM are currently not fully understood.

The essential amino acid Tryptophan (Trp) undergoes degradation via two distinct pathways (i.e. the serotonin and kynurenine pathway). The kynurenine pathway is of particular interests as its key step is induced by pro-inflammatory mechanisms via the immunoregulatory enzyme indoleamine-2,3-dioxygenase (IDO). Importantly, secondary catabolites downstream of kynurenine (such as kynurenic acid and quinolinic acid) are well-known immunosuppressants and/or neurotoxins. IDO itself plays key roles both in the regulation of inflammation and in development of immunological tolerance for example in pregnancy,[15] tumor-related immune escape,[16–19] kidney or vascular disease including stroke,[20,21] neurodegenerative disease,[22,23] severe infections/severe sepsis,[24–26] inflammation-induced hypotension/shock,[27] or in animal models of asphyxia.[28] Few previous data show that activation of Trp catabolism may occur early after CA in humans[29,30] and point to the fact that kynurenine pathway catabolites may be associated with severity of post-cardiac arrest shock, early death and/or poor long-term functional outcome. However, single blood samples after ICU admission were mostly investigated and further studies are necessary to elucidate the course of IDO-activation and levels of respective catabolites over time. Moreover, few previous experimental data show that IDO-activity may be influenced by hypoxia and respective data indicate an impaired antimicrobial response under hypoxic conditions.[31–33]

In the current study we aim to characterize the longitudinal course of both IDO-activity and Tryptophan degradation products (i.e. kynurenine catabolites) in patients with cardiac arrest receiving target temperature management. This is performed as, to the best of our knowledge, a potential influence of core body temperature on Tryptophan catabolism and IDO-activity was not investigated so far. In an effort for a better characterization, an observational analysis with a blinded endpoint design was performed.

Material/methods

Between May 2010 and May 2011, a total of 20 critically ill comatose survivors from cardiac arrest were assessed in a monocentric observational trial with a blinded endpoint assessment. The present study was performed as a substudy of a prospective analysis on plasma choline in CA patients.[34] The initial overall cohort consisted of 26 patients, of which 6 patients were excluded from the analysis due to death within 24 h following admission or missing informed consent (please refer to exclusion criteria). In all 20 patients entering the analysis, written informed consent was provided by a court-ordered legal representative. All patients were treated at the intensive care units of a tertiary care academic center (Charité University Medicine, Berlin, Germany). Inclusion criteria were met if patients remained unconscious after successful resuscitation from cardiac arrest regardless of initial rhythm or suspected etiology. Patients were excluded in case of age <18 years, presence of severe anemia defined by hemoglobin levels <8 g l^{-1}, and death within 24 h of admission.

Target temperature management (TTM) to achieve core body temperatures of 32–34 °C was applied in all cardiac arrest patients directly following ICU admission and was applied for a total period of 24 h, followed by 24 h of controlled rewarming (0.25 °C per hour) to normothermia (i.e. 37 °C core body temperature). Hypothermia defined as 32–34 degrees Celsius core body temperature was reached in 100% of cases by using a computer feedback surface cooling device (ArcticSun, C.R. BARD, Colorado, USA). The study was performed in accordance to the *Declaration of Helsinki* and IRB approval was achieved by the local ethical committee of the Charité Universitätsmedizin Berlin, Berlin, Germany).

Assessment of blood samples

Blood sampling was performed at the following 16 time points post ROSC: 30 min, 60 min, 90 min, 120 min, 150 min, 180 min, 4 h, 6 h, and 12 h/24 h (hypothermia phase), 48 h, 72 h, 96 h, at day 5, day 6, and day 7. Samples were drawn from arterial lines or central venous catheters and aliquots were stored at −80 °C until analysis. In total, out of the 320 intended samples, 270 samples (i.e. 84%) were available for assessment. In addition to analysis of the respective course of Tryptophan catabolites over the observational period, samples were grouped and analyzed for at what specific time respective blood samples were drawn (i.e. "before" established target temperature, "on" established target temperatures, and "after" hypothermia).

Temperature monitoring

Temperature was assessed using an esophageal temperature probe (Level 1 Esophageal/Rectal Temperature Probe, Smiths Medical ASD Inc., St. Paul, MN, USA) with online reporting and documentation in the digital patient data management system. Following assessment of temperature, automatic temperature adjustment was performed by the computerized surface cooling device in an effort to achieve target temperatures.

Assessment of Tryptophan catabolites and IDO activity

Assessment of the essential amino acid Tryptophan (Trp) and respective catabolites was performed from plasma samples with respective investigators being blinded to clinical data. Assessment of Tryptophan and Tryptophan catabolites was performed in accordance to the procedure by Zhu et al.[35] and as reported elsewhere.[16] In detail, commercial Trp, kynurenine (Kyn), kynurenic acid (KynA), quinolinic acid (QuinA), 5-hydroxy Tryptophan (OH-Trp), 3-hydroxy anthranilic acid (3-OH ANA), serotonin (Ser), phenylalanine (Phe) (all Sigma-Aldrich, St. Louis, USA), deuterium labeled compounds Kyn-d6, KynA-d5, Phe-d5, Trp-d5 (all Cambridge Isotope Laboratories, Andover, MA, USA), water (Optima MS grade, Fisher Scientific, Waltham, USA) and acetonitrile (Optima grade, Fisher Scientific, Waltham, USA) were used. Frozen aliquots were thawed prior to analysis. 100 μl sample volume was added to a deuterated internal standard mixture (50 μl) of equal volumes of Kyn-d_6, Kyn A-d_5, Phe-d_5, Trp-d_5. After shaking, acetonitrile was added and left over night at −20 °C for precipitation. Samples were centrifuged. The supernatant was dried under vacuum centrifugation and reconstituted with 100 μl H_2O/acetonitrile (95%/5%). A Waters Acquity UPLC-TQD system (Milford, MA, USA) was equipped with an electrospray ion source using MRM detection in a positive ion mode. The following transitions of mass-to-charge ratios (m/z) of 205/188 for Trp, 210/193 for Trp-d_5, 209/192 for