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Short paper

Low serum Kallistatin level was associated with poor neurological outcome of out-of-hospital cardiac arrest survivors: Proteomics study

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ABSTRACT

Aim of the study: To identify proteins of which depletion are associated with the poor 6-month neurological outcome of out-of-hospital cardiac arrest survivors.
Methods: Seven healthy volunteers and 34 out-of-hospital cardiac arrest survivors admitted to the intensive care unit (ICU) and underwent targeted-temperature management were enrolled. According to the 6-month cerebral performance category (CPC) scale, patients were divided into the good (CPC 1–2) and poor (CPC 3–5) outcome groups. Blood samples were obtained at 0, 24, and 72 h after admission to the ICU.
Results: With proteomic approaches, we found 23 proteins that showed group-differences between the sera pooled from 7 study groups: healthy volunteers, the good outcome groups (0, 24, and 72 h), and the poor outcome groups (0, 24, and 72 h). We selected 7 candidate proteins of which intensities were different between the good and poor outcome groups (> 2-fold change) and excluded 5 proteins related to haemolysis or remaining high abundant proteins. To confirm the 2 identified proteins: retinal dehydrogenase 1 and Kallistatin, we performed enzyme-linked immunosorbent assay with individual serum. Finally, old age (odds ratio = 1.055; 95% confidence interval, 1.002–1.112; $p = 0.043$) and low serum kallistatin level at 0 h (odds ratio = 0.784; 95% confidence interval, 0.618–0.995; $p = 0.046$) were independently associated with the poor 6-month neurological outcome.
Conclusion: The depletion of serum kallistatin at admission to the ICU was associated with the poor neurological outcome of out-of-hospital cardiac arrest survivors.

Introduction

Cardiac arrest frequently results in neurological disability [1,2]. However, no pharmacological agents are available to improve neurological outcomes of cardiac arrest survivors [3]. During the past decades, many biomarkers have been developed. Most of them, such as neuron specific enolase and S-100 protein, are overexpressed in patients with poor neurological outcome [4–6]. They are only markers for recent or present neuronal injuries, and thus, their elimination or inhibitor use have not been considered as therapeutic strategies [4–6]. For the development of pharmacological agents, candidate proteins of which depletion is significantly associated with poor neurological outcome and their supplement may improve neurological outcome should be identified [7,8].

Proteomics is a peptide screening method to identify candidate proteins associated with pathological conditions by investigating all of the integrated proteins rather than an individual protein [9,10]. Therefore, we hypothesized that with proteomic approaches, we could compare the changes in serum proteome profiles between cardiac arrest survivors with good and poor neurological outcomes and could identify candidate proteins of which depletion are significantly associated with poor neurological outcome.

Our aim was to identify proteins of which depletion was associated with the poor 6-month neurological outcomes of cardiac arrest survivors.

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Methods

Subjects

This study was conducted in a 12-bed intensive care unit (ICU) of a tertiary referral hospital and was approved by the Institutional Review Board of our institute (IRB No., 1601-127-739). The repository protocol is registered at ClinicalTrials.gov (NCT01670383).

We enrolled healthy volunteers and out-of-hospital cardiac arrest survivors who were admitted to the ICU for post-resuscitation care from March 2013 to December 2015. Written informed consent was obtained from healthy volunteers and each patient's next of kin.

Exclusion criteria were pregnancy, age < 18 years old, non-cardiac causes including trauma, intoxication, drowning, and hanging, insufficient blood samples, presence of an advanced directive to withhold or withdraw life-sustaining treatment, no informed consent, contraindications to targeted-temperature management (TTM) including haemorrhage, fatal arrhythmia, haemodynamic instability, or sepsis, or follow-up loss. According to the 6-month cerebral performance category (CPC) scale [11], the patients were divided into good (CPC 1–2) and poor outcome (CPC 3–5) groups.

Post-resuscitation care and data collection

All of the enrolled patients were admitted to the ICU within 30 min post-return of spontaneous circulation (ROSC) and were treated with standardized post-resuscitation care including TTM using an external cooling device (Arctic Sun, Bard, Inc., Louisville, CO), according to the current guideline [3]. Core temperatures were cooled to 32–34 °C within 4 h post-ROSC and maintained for 24 h, then, were rewarmed at a rate of 0.25 °C/h and maintained at less than 37 °C for 72 h.

At admission to the ICU, we collected demographic data. Blood samples were obtained from the healthy volunteers and patients at 0, 24, and 72 h after admission to the ICU. Blood samples were allowed to clot at room temperature for 20 min and were centrifuged at 1258 g for 10 min at 4 °C. The separated sera were stored at −80 °C until further analyses.

Protein pooling

Obtained sera were divided into 7 groups and pooled as follows: (i) 7 healthy volunteers, (ii) 19 patients with poor outcome at 0 h, (iii) 19 patients with poor outcome at 24 h, (iv) 19 patients with poor outcome at 72 h, (v) 15 patients with good outcome at 0 h, (vi) 15 patients with good outcome at 24 h, and (vii) 15 patients with good outcome at 72 h. From each group, 3 mg of proteins were pooled after bicinchoninic acid (BCA) quantification.

High abundant protein depletion

Human albumin, immunoglobulin G (IgG), antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, and transthyretin were eliminated using the Multiple Affinity Removal System (MARS) depletion technique.

Liquid chromatography–mass spectrometry

After high abundant protein depletion, 200 μg of protein from each group was lysed according to the 8 M Urea In-solution Digest Protocol, reduced with 10 mM Dithiothreitol (DTT), alkylated with 30 mM Iodoacetamide (IAA), digested at 37 °C overnight with 1% trypsin, and desalted using a Harvard spin column [12]. Liquid chromatography and mass spectrometry (LC–MS) were performed using Thermo EASY-nLC 1000 and Thermo Q-Exactive. Then, label-free quantification and protein identification were performed using MaxQuant software for proteomics [13]. All procedures were performed in triplicate.

Confirmative enzyme-linked immunosorbent assay (ELISA)

We performed ELISA with each individual sample in duplicate using a Human Retinal dehydrogenase 1 (ALDH1A1) ELISA Kit (abx250240, Abbexa Ltd., Cambridge, UK) and a Human Serpin A4/Kallistatin DuoSet ELISA kit (DY1669, R&D Systems, Minneapolis, MN).

Statistics

Demographic data were analyzed using Student's *t*-test, Chi-square test, or Fisher's exact test as appropriate. To find out candidate proteins, (i) we used – log analysis of variance (ANOVA) p values > 9 between the 7 groups (= ANOVA p values < 1×10^{-9} calculated by the Perseus programme of MaxQuant) as the threshold for filtering and (ii) we selected proteins of which expressions differed by more than a 2-fold change between the good and poor outcome groups at any time point. Serial ELISA data were analyzed using the stepwise logistic regression analysis (with an entry level of 0.05 and a stay level of 0.05).

Next, to investigate which parameters are independently associated with the poor 6-month neurological outcome of cardiac arrest survivors, the multiple logistic regression analysis was applied. P values of < 0.05 were considered to be statistically significant, and the significance levels quoted are two sided. The statistical analyses were conducted using SPSS version 21.0 for Windows (SPSS, Chicago, IL).

Results

Patients' characteristics

Among 34 enrolled patients, 19 were categorized in the poor outcome group and 15 in good outcome group (Supplemental Fig. 1). Patients in the poor outcome group were older and underwent less coronary angiography (CAG) than those in the good outcome group (Supplemental Table 1). There were no significant group-differences in other variables including core temperatures during TTM (Supplemental Fig. 2).

Candidate protein selection with pooled samples

When we compared the 7 pooled groups, 23 proteins showed more than 9 of – log ANOVA p-values (Fig. 1A and Supplemental Table 2), and 7 proteins showed differences among intensities between the good and poor outcome groups of more than a 2-fold change (Table 1 and Fig. 1B). Among them, 5 proteins associated with haemolysis which might occur during the blood preparing procedures or the remaining high abundant proteins were excluded, and 2 proteins were finally selected (Table 1).

Confirmative ELISA with individual samples

Among the serial serum protein levels measured by ELISA, low Kallistatin level at 0 h was significantly associated with the poor 6-month neurological outcome (odds ratio = 0.445; 95% confidence interval, 0.241–0.822; p = 0.010) (Table 2 and Fig. 1C).

In multivariable analysis, old age (odds ratio = 1.055; 95% confidence interval, 1.002–1.112, p = 0.043) and low serum Kallistatin level at 0 h (odds ratio = 0.784; 95% confidence interval, 0.618–0.995, p = 0.046) were independently associated with the poor 6-month neurological outcome of cardiac arrest survivors (Table 2).

Discussion

In the present study, we found that the low serum Kallistatin level was independently associated with the poor 6-month neurological