Resuscitation 85 (2014) 527–532

Contents lists available at ScienceDirect

Resuscitation

journal homepage: www.elsevier.com/locate/resuscitation

Ischaemic brain damage after cardiac arrest and induced hypothermia–a systematic description of selective eosinophilic neuronal death. A neuropathologic study of 23 patients

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ARTICLE INFO

Article history:
Received 20 May 2013
Received in revised form
21 November 2013
Accepted 24 November 2013

Keywords:
Cardiac arrest
Hypothermia
Brain damage
Neuronal death

ABSTRACT

Purpose: Although well characterized in animals, brain damage in humans treated with hypothermia after cardiac arrest has not been systematically explored. In this study we aimed to describe the characteristic trait of selective eosinophilic neuronal death (SEND), and its correlation with time to return of spontaneous circulation (ROSC) in cardiac arrest patients who died after hypothermia treatment and were referred for autopsy.

Methods: Brain autopsy microscopic slides and clinical data were gathered from 23 non-survivors of cardiac arrest who were treated with hypothermia. Based on the percentage of eosinophilic neurons, a damage score 0–4 was given in 6 brain regions, and a total damage score was calculated. The damage score was correlated with time to ROSC and with neuron-specific enolase (NSE) in peripheral blood at 48 h post arrest.

Results: Hippocampus had the highest damage score with a median of 3 (inter-quartile range 2–4) while the brainstem had the lowest median damage score of 0 (0–2). Total damage score showed the best correlation with time to ROSC (Spearman Rho = 0.66). Serum NSE values >33 μg/L ($n = 6$) was associated with significantly higher mean damage score than NSE <33 μg/L ($n = 9$) ($p = 0.002$).

Conclusion: This is the first study to systematically describe regional SEND in patients treated with hypothermia after cardiac arrest. Hippocampus was the most vulnerable region whereas the brainstem was the most resistant. Although not directly compared here, the regional pattern of SEND seems not to be altered by hypothermia treatment, but maintains its profile distinctive for cardiac arrest pathogenesis.

1. Introduction

In a patient with sudden cardiac arrest and return of spontaneous circulation (ROSC), the brain suffers from transient global ischaemia. In this situation, a typical histopathologic pattern of ischemic brain damage is recognized as selective neuronal necrosis, as defined in the 1980's.[1] The term necrosis is however somewhat misleading, therefore the strictly morphological denomination selective eosinophilic neuronal death (SEND) will be used. The particular ischaemic damage of SEND stands in contrast to the event of a focal cerebrovascular insult where all cell types, including neuroglia and endothelial cells, are damaged.[1] When exposed to global ischaemia, neurons die within hours to days.[2,3] This is known as delayed neuronal death and provides a therapeutic window for intervention. Induced hypothermia has been shown to mitigate neuronal death in the hippocampal CA1 zone[4] and cerebellar Purkinje cell layer in rat[5,6] as well as in gerbil[7] after cardiac arrest. In humans hypothermia has been demonstrated to improve neurologic outcome[8,9] and survival[8] after cardiac arrest and is now recommended treatment in the European Resuscitation Council Guidelines.[10]

The concept of selective vulnerability suggests that a global ischaemic insult to the brain results in a variable degree of damage depending on region. A study on normothermic dogs described the

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http://dx.doi.org/10.1016/j.resuscitation.2013.11.022

Table 1
Pre-existing disease condition.

Pre-existing condition[a]	N	(%)
Hypertension	9	39
Lung disease	5	22
IDDM	4	17
Coronary illness	4	17
Heart failure	2	9
Renal disease	2	9
Neurologic disease[b]	2	9
Liver disease	1	4

IDDM = insulin dependent diabetes mellitus.
[a] Defined as: medicating for and/or attending control-visits regularly for the condition.
[b] Diabetic polyneuropathy in both cases.

Table 2
Brain regions evaluated microscopically for assessment of SEND.

Regions scored	Neurons evaluated with X10 objective
Hippocampus	The CA[a]1, CA2, CA3, CA4 and the dentate gyrus
Cerebellum	Purkinje cells
Neocortex	Parts of the frontal and parietal cortex judged representative for the average degree of damage
Basal ganglia	The caudate nucleus, the mid-putamen and globus pallidus
Thalamus	Neurons in the thalamus, any part available on the sections
Brainstem	The mesencephalon and pons

[a] CA = Cornu Ammonis.

regional progression of ischaemic neurons with increasing duration of cardiac arrest.[11] The regions most vulnerable to ischaemia in that study included the hippocampus, the cerebellum and the caudate nucleus, whereas the midbrain, medulla and pons were relatively resistant. Human neuropathologic studies of cardiac arrest victims have shown various degrees of ischaemic damage in the hippocampus, cerebellum, neocortex and basal ganglia[2,12,13] and a relative invulnerability of the brainstem.[12,13] However, the neuropathology in humans treated with hypothermia after cardiac arrest has been only briefly described.[14,15] To our knowledge, there are no systematic studies on this topic.

The serum biochemical marker neuron-specific enolase (NSE) has been used for prognostication of outcome after cardiac arrest[16] and was shown to correlate with other prognostic markers.[14] The level of serum NSE may theoretically correlate with degree of neuronal damage and as such serve to corroborate the evaluation.

Pre-existing conditions such as diabetes and hypertension may possibly affect the neuropathological findings. Hypertension affects the auto-regulation of blood flow in the brain and may thus affect the ischaemic damage after cardiac arrest.[17] Hyperglycemia has been shown to exacerbate ischaemic damage in cerebral vascular insults[18] and diabetes mellitus is a known risk factor for worse outcome in cardiac arrest patients.[19]

The main goal of this study was to describe the severity and regional distribution of the particular ischaemic brain damage of SEND in a population of deceased cardiac arrest patients treated with hypothermia. A second goal was to analyze the correlation between regional extent and severity of SEND and time to ROSC. Furthermore, we aimed to investigate a possible correlation between brain damage and serum NSE at 48 h and to search for predominant pre-existing morbidities.

2. Study group and methods

2.1. Patients

We retrospectively analyzed histopathological brain sections from 23 cardiac arrest non-survivors treated with TH at the Skane University Hospital in Lund. Patients with clinical data and sufficient available neuropathology at the time of the study were studied. From this group of cases, a subset was briefly presented earlier.[14,15] The group consisted of 18 men and 5 women, mean age being 61.6 ± 14.7 years. Pre-existing conditions are shown in Table 1. The cause of the cardiac arrest was primary cardiac in 14 cases, extra-cardiac in 3 cases and unknown in 6 cases. 17 (74%) were out-of-hospital cardiac arrests and median time to ROSC was 25 (inter-quartile range (IQR) 20–35) min. Median time to death was 127 (IQR 107–150) h with shortest time being 38 h, all patients having reached normothermia before death. The cause of death was classified, in line with procedures described in an earlier work,[15] as brain injury in 22 (96%) patients and cardiac disorder in 1 (4%) patient. One patient was declared brain dead according to Swedish legislation. In the remaining 21 individuals with brain injury, life-supporting treatment was withdrawn after delayed neurological prognostication.[15]

Similar to earlier studies on hypothermia treatment, the core body temperature was lowered to 33 °C for 24 h and thereafter increased by 0.5 °C/h until normothermia was reached.[8,20] Clinical data were prospectively collected from the patients' hospital medical records and ambulance records. In 15 patients NSE values (Liaison® DiaSorin AB, Sundbyberg, Sweden) sampled 48 h after cardiac arrest were available.

2.2. Histopathological evaluation

The brains were fixed in 4% formaldehyde solution and sectioned in a coronal plane. Six specific regions (the hippocampus, cerebellum, neocortex, basal ganglia, thalamus and brainstem) were selected for microscopic evaluation and stained with hematoxylin-eosin. The regions were selected based on earlier studies in humans[2] and animals[11] and on our own clinical experience from previous cardiac arrest cases. An X10 objective was used for quantification of SEND, while an X4 objective was used for screening and judgement of representativity of SEND levels in the regions assessed with X10. The regions examined included a midsection of the hippocampus, the basal ganglia and the thalamus from one hemisphere as well as the brainstem. These areas were examined using an X4 objective and at least one microscopic field of view within each area listed in Table 2 was examined using a X10 objective. In each region, the subregion harboring the highest degree of damage was chosen for quantification. The neocortex was examined with four fields of view at X4, from the interhemispheric midline over the convexity in lateral direction, including at least one sulcus. One field of view judged representative with regard to SEND levels was quantitatively examined with an X10 objective. The cerebellum was similarly assessed with four fields of view at X4 and one field at X10. Altogether 6 brain regions were assessed, comprising 13 subregions (Table 2). All ratings were conducted blinded to clinical data.

Neuronal death in cardiac arrest brain ischaemia, SEND, was defined based on the level of microstructural alterations, connoting an eosinophilic change of neurons with reduced discernible intracellular components. The cell nucleus and the entire cell becomes pycnotic, shrunken, the cytoplasm-nuclear border is indistinct or lost, as is also the nucleolus. The surrounding neuropil is rather unchanged, with no reactive cells and no edema related to the neuronal change. Altogether, these changes have earlier been shown to signify irreversible ischaemic neuronal damage.[1,2,11] The damage in each subspecified region, assessed with an X10 objective, was graded in a numerical severity score, modified from earlier studies.[2,11] It is here referred to as the SEND damage score, and based on the percentage of devitalized, eosinophilic (red) neurons