APPLICATION OF A FOUR-DIMENSIONAL MATHEMATICAL MODEL IN THE ESTABLISHMENT OF AN EARLY POST-BURN CEREBRAL OEDEMA MODEL IN SEVERELY BURNED DOGS

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SUMMARY. The aim of this study was to explore the spatiotemporal development of cerebral oedema in the early stage of severe burn (50% TBSA, third degree), using a four-dimensional (4D) mathematical model. Twenty-six male mongrel dogs were randomly divided into control and 6, 12, 18, and 24 post-burn hour (PBH) groups. The manifestation of magnetic resonance imaging (MRI) and histopathology, changes of brain water content, and intracranial pressure were observed in each group respectively. A 4D mathematical model was established on the basis of the results of MRI scanning. Two turning points (6 and 18 PBH) and three phases of pathological change were displayed by the 4D mathematical model of cerebral oedema in the early stage of severe burn. The first phase was in the subclinical period, and effective treatment should therefore be performed as quickly as possible in order to prevent deterioration of post-burn cerebral oedema. The second phase (6-18 PBH), with pathological characteristics of cytotoxic cerebral oedema, was in the apoptosis period. The third stage (18-24 PBH) was the danger period of cerebral oedema. Intracranial pressure increased rapidly owing to the limitation of the cranial cavity. As a result, cerebral hernia could easily occur. An S-shape curve in the pathological process of cerebral oedema occurred in the early post-burn stage following severe burn.

Introduction

Post-burn cerebral oedema is one of the most important complications in severely burned victims, with a complicated pathogenesis that develops more quickly and with a more severe prognosis when the burn degree is more severe. Like other diseases, early post-burn cerebral oedema develops with a time lapse. Consequently, changes in the three-dimensional structure are studied over time. In this study, a four-dimensional (4D) mathematical model was established in accordance with dynamic pathomorphological observations and image examination of brain tissue in burned dogs with early post-burn cerebral oedema, with a view to exploring the pathogenesis of cerebral morphological changes during the development of early post-burn cerebral oedema.

Materials and methods

Preparation before burn and method of burn injury

Twenty-six healthy male mongrel dogs weighing 11.5 ± 1.5 kg were randomly divided into control (C, n = 6) and injury (I, n = 20) groups. The animals were first systematically anaesthetized with 3% pentobarbital sodium (30 mg/kg) and then depilated. The dogs in I group were further divided averagely into groups according to post-burn time, i.e. 6, 12, 18, and 24 post-burn hour (PBH, n = 5 per group) groups. The dogs in I group received a napalm flame burn on the back, causing a 50% TBSA third-degree burn. A 5% glucose solution was infused intravenously from PBH 6, according to the Parkland formula, establishing the model of early post-burn cerebral oedema in group I dogs. Group C dogs underwent all the procedures except burn injury.

Indices of observation after burn

MRI scanning

0.2T open-style permanent magnet MR equipment was employed in the MRI scanning. The spin-echo sequence was made routinely with T1WI (TR/TE as 560/30 ms) and T2WI (TR/TE as 6000/114 ms). Nine layers were harvested in each sequence. Image analysis (regions of interest [ROI]) was carried out on both sides of the cortices of the frontal lobe, parietal lobe, and cerebellum and nuclei basales areas (0.3 x 0.3 cm/each ROI). The average MR signal intensity of ROI in all layers was measured, and the average signal intensity in normal corresponding subcutaneous tissue was determined at the same time. The ratio
of the two intensities was set to be the standardized signal intensity ratio (SIR).

Pathomorphological examination

5 x 5 x 5 mm of tissue samples were harvested for HE staining and LM examination from the ROI, i.e. both sides of the cortices of the frontal, parietal, cerebellum, bulb, and nuclei basales lobes. In addition, 1 x 1 x 1 mm of brain tissue was examined by transmission EM.

Determination of brain water content (BWC)

Brain tissue (0.5 g) was sampled from all ROI for the determination of wet weight. The tissue was baked at 80 °C in an electric oven at constant temperature for 72 h, after which the tissue weight was unchanged (less than 0.2 mg weight difference between two times of weighing). BWC was calculated by the Elliot formula (BWC = [wet weight-dry weight]/wet weight x 100%).

Determination of intracranial pressure (ICP)

The transduction tube was inserted into the subarachnoid space via the interspace between third and fourth lumbar vertebrae and connected to a Viridia all-round monitor by pressure sensor. The change in ICP value at all time spots was detected.

Statistical analysis

ANOVA and t test were employed in the data process. All the data were expressed as ± S.

The four-dimensional mathematical model was established accordingly

Data collection. All the images were fed into a computer. The cross-section areas of brain tissue and cranial cavity at all layers were calculated and processed statistically. The average value of brain tissue areas (S\textsubscript{i,j}) and the average value of cranial cavity areas (S\textsubscript{max}) were calculated (Table I).

Table I - Average value of brain tissue areas (S\textsubscript{i,j}) and cranial cavity areas (S\textsubscript{max})

<table>
<thead>
<tr>
<th>Layer (cm)</th>
<th>Average value of brain tissue areas (S\textsubscript{i,j})</th>
<th>Cranial cavity areas (S\textsubscript{max})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>6h</td>
</tr>
<tr>
<td>0.0</td>
<td>2.800</td>
<td>2.830</td>
</tr>
<tr>
<td>1.2</td>
<td>4.100</td>
<td>4.410</td>
</tr>
<tr>
<td>1.8</td>
<td>4.870</td>
<td>4.900</td>
</tr>
<tr>
<td>2.0</td>
<td>5.110</td>
<td>5.180</td>
</tr>
<tr>
<td>2.4</td>
<td>5.030</td>
<td>5.040</td>
</tr>
</tbody>
</table>

Calculation of brain volume. Spline interpolation from mathematical software Mathematica 3.0 was employed to construct the cubic interpolation polynomial of all layers: s = f(x), so that the relative actual areas of brain tissue at different layers could be obtained. Simpson formula 1 for approximate calculation of definite integral was employed to calculate the canine brain volume (V\textsubscript{i}) and cranial cavity volume.

Formula 1

\[
V_j = \frac{3}{(2n)} \left[ \frac{S_{i=1}}{2n} + \frac{S_{i=2}}{2n} + \frac{S_{i=3}}{2n} + \ldots + \frac{S_{i=2n}}{2n} \right]
\]

where 2n means the number of subintervals obtained from dividing the interval [0, 3.2].

Fabrication of the volume-time diagram of curve

According to Mathematica 3.0, the volume V (cm\textsuperscript{3}) was set to be the vertical ordinate and the time t (h) the horizontal coordinate. The volume-time curve (4D mathematical model) was drawn in accordance with the change of canine brain tissue volume along with that of time.

Results

Experimental results

MRI scanning

Control exhibited normal cerebral morphology and signals. The brain tissue in the 6 PBH group presented no difference from that of control. Diffuse swelling of the brain parenchyma was identified at T\textsubscript{1}WI in two dogs at 12 PBH, in three at 18 PBH, and in four at 24 PBH. The enhanced signals of the brain parenchyma and blurred eptocinerea and white matter dividing was found at T\textsubscript{2}WI in two dogs in the 24 PBH group. T\textsubscript{1}WI SIR in the 24 PBH group decreased by 10.39% compared to that of the control group (p < 0.05), while the T\textsubscript{2}WI SIR increased by 8.29% (p < 0.5) (Table II).

Table II - Changes of mean SIR with time lapse (± S)

<table>
<thead>
<tr>
<th>Group</th>
<th>SIR of T\textsubscript{1}WI</th>
<th>SIR of T\textsubscript{2}WI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>1.64 ± 0.09</td>
<td>1.45 ± 0.16</td>
</tr>
<tr>
<td>PBH 6</td>
<td>1.66 ± 0.11</td>
<td>1.46 ± 0.12</td>
</tr>
<tr>
<td>PBH 12</td>
<td>1.54 ± 0.13</td>
<td>1.46 ± 0.12</td>
</tr>
<tr>
<td>PBH 18</td>
<td>1.48 ± 0.12</td>
<td>1.48 ± 0.12</td>
</tr>
<tr>
<td>PBH 24</td>
<td>1.47 ± 0.15*</td>
<td>1.57 ± 0.15*</td>
</tr>
</tbody>
</table>

p < 0.05 vs control group
Pathomorphological examination

The brain tissue in control sections was normal by LM and EM. Capillary hyperaemia was found in the 6 PBH group, with an irregular capillary lumen and widened pericapillary interspace. The endothelial cells of the capillaries revealed hypertrophy by EM, with swelling end-process of the astrocytes. The endothelial cells within the capillaries collapsed in the 12 PBH group, with partial loose intercellular space and additional widening of the pericapillaries. Also, vacuolar degeneration and neurotropic phenomena were found in part of the nerve cells. In the 18 PBH group, the derangement and the crista break of the mitochondria in the capillary endothelia were identified with nuclear swelling and an irregular capillary basement membrane. The astrocytic end-process exhibited vacuolar swelling.

The change of BWC

BWC at 12 PBH was 78.31% and increased until 18 and 24 PBH, respectively 79.86% and 81.94% ($p < 0.01$) (Table III).

<table>
<thead>
<tr>
<th>Group</th>
<th>BWC (%)</th>
<th>ICP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>74.67 ± 0.55</td>
<td>2.10 ± 0.15</td>
</tr>
<tr>
<td>PBH 6</td>
<td>76.65 ± 0.42</td>
<td>2.22 ± 0.25</td>
</tr>
<tr>
<td>PBH 12</td>
<td>78.31 ± 1.13*</td>
<td>2.62 ± 0.23</td>
</tr>
<tr>
<td>PBH 18</td>
<td>79.86 ± 0.87**</td>
<td>3.17 ± 0.19***</td>
</tr>
<tr>
<td>PBH 24</td>
<td>81.94 ± 0.49**</td>
<td>3.24 ± 0.05**</td>
</tr>
</tbody>
</table>

$p < 0.05$, ** $p < 0.01$ vs control group

ICP increased significantly at 18 PBH and 24 PBH ($p < 0.01$) (Table III), compared with that of control.

Establishment of the 4D mathematical model

The brain tissue volume increased gradually and reached its peak value at 24 PBH. The cranial cavity volume in control was taken as the standard cranial cavity volume owing to its constant volume in all groups (Table IV).

<table>
<thead>
<tr>
<th>Group</th>
<th>BTV [V(cm$^3$)]</th>
<th>CCV (cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.80 ± 0.11</td>
<td>15.93 ± 0.26</td>
</tr>
<tr>
<td>PBH 6</td>
<td>14.07 ± 0.21</td>
<td>15.93 ± 0.33</td>
</tr>
<tr>
<td>PBH 12</td>
<td>14.78 ± 0.32</td>
<td>15.92 ± 0.53</td>
</tr>
<tr>
<td>PBH 18</td>
<td>15.40 ± 0.44*</td>
<td>15.93 ± 0.51</td>
</tr>
<tr>
<td>PBH 24</td>
<td>15.52 ± 0.41</td>
<td>15.93 ± 0.12</td>
</tr>
</tbody>
</table>

$p < 0.05$ vs control group

A curve was drawn according to the relationship between post-burn brain swelling volume and time lapse (Fig. 1).

Discussion

It is rare to see reports of the pathomorphological changes of internal organs and the development of these changes in 4D mathematical models.\cite{14} Brain morphology alters during the pathological development of cerebral oedema in the early post-burn period. The mathematical model employed in the description of this change was helpful for the observation of the macroscopic change in brain tissue over a lapse of time. The internal relationship between the changes of brain tissue morphology and time points was analysable by mathematical expressions. The curve not only set the spatial sites of all the time points but also accurately described the dose-effect relationship and the time-effect relationship between brain tissue change and time lapse. The establishment of the curve also promoted the morphological study of cerebral oedema from 3D to 4D.

The spline interpolation from the mathematical software of Mathematica 3.0 was employed in the study to better simulate the morphological changes of early post-burn cerebral oedema. A scattered diagram was drafted from the actual area data of nine layers at all time points, every two points of which were curve-fitted by $ax^3 + bx^2 + cx + d$. The curve connecting all the nine points was obtained as: $s = f(x)$. The actual area of all-thickness brain tissue was calculated by the curve $s = f(x)$. Each layer scanned by MRI of the brain was further divided into four layers, every layer being 1 mm thick. The Simpson formula was employed to calculate the actual volume at each time point according to the actual area of all time points.
The relationship between post-burn time and actual brain volume was described by the curve produced by Mathematica 3.0. The curve reflected the relationship between post-burn oedematous brain volume and time.

The curve of brain volume vs post-burn time exhibited an S-shape, which indicated the two turning points (TP) and three periods of the change of cerebral morphology during early post-burn time (24 PBH). The first TP appeared at 6 PBH. The slope function of the curve after 6 PBH increased manifestly. The second TP came at 18 PBH, after which the slope function became gentler.

**The first period (within 6 PBH)**

The victims reached the shock stage after severe burn injury. There were no evident pathological changes in the brain tissue, which on MRI scanning exhibited normal volume and shape, with normal structure; however, we observed capillary hyperaemia as well as widened pericapillary interspace and mild ischaemic changes of the endothelia and nerve cells. This implied that there was a slight change in the brain blood barrier in brain tissue within the local area. BWC at this time increased by only about 2.65%, while intracranial pressure increased by 5.71%. This period was the subclinical phase of early post-burn cerebral oedema pathology. These data suggest that early effective procedures are necessary to prevent the development of cerebral oedema.

**The second period (6-18 PBH)**

The 6-18 PBH period was the peak time of post-burn fluid exudation. The slope function of the curve of brain volume vs post-burn time increased significantly, which reflected the rapid development period of post-burn cerebral oedema. The ratios of cerebral pathomorphological and signal changes clearly increased when examined by MRI in this period, and the pathological changes were aggravated in intensity and extent compared to those at 6 PBH. Also, the capillary endothelia collapsed and the conjunctive interspace loosened, with vascular changes in the nerve cells, a 6.95% increment of brain water content, and a 50.95% increment of ICP, indicating that a tissue pathology had developed in which cytotoxic cerebral oedema characterized by intracellular swelling was evident. The period was considered key in the development of early post-burn cerebral oedema and therefore in its clinical management.

**The third period (18-24 PBH)**

This period featured a nearly gentle slope function of the curve of brain volume vs post-burn time, which reflected the fact that the change in brain volume became stable along with the lapse of post-burn time, compared to that of the second period. Cerebral oedema was more obvious by MRI. The pericapillary space and the intracellular oedema of the nerve cells appeared more evident. The glial cells showed vacuolar swelling. The brain tissue water content increased by 9.74% and ICP increased by 54.29%, indicating that brain volume did not increase significantly as post-burn time lapsed, owing to the rigidity of the skull, which led to the curve slope function becoming gentle even if the disease was still progressively developing. However, ICP increased sharply, which contributed to the formation of cerebral hernia. The third period of early post-burn cerebral oedema is critical for the development of the disease and for saving a patient’s life.

**Conclusion**

This is just a preliminary attempt to investigate the rules and the mechanism of the development of early post-burn cerebral oedema pathomorphology using a four-dimensional mathematical model. This could be very useful for the understanding of the pathogenesis of post-burn internal organ injuries (including the brain) and for their management.

RÉSUMÉ. Dans cette étude les Auteurs se sont proposés d’explorer le développement spatio-temporel de l’œdème cérébral dans la phase précoce des grandes brûlures (50% surface totale corporelle brûlée, troisième degré) utilisant un modèle mathématique à quatre dimensions (4D). Vingt-six chiens bâtards mâles ont été divisés au hasard en groupe témoin et groupe post-brûlure à 6, 12, 18 et 24 h (PBH). Les Auteurs ont observé dans chaque groupe respectivement les résultats de l’imagerie par résonance magnétique (IRM) et de l’histopathologie, les modifications du contenu hydrique, et la pression intracrânienne. Un modèle mathématique à 4D a été créé sur la base des résultats du scanning IRM. Le modèle mathématique à 4D de l’œdème cérébral dans la phase précoce a démontré deux moments décisifs (PBH 6 et 18) et trois phases de changement pathologique. La première phase s’est vérifiée pendant la période subclinique et pour cette raison il faut pratiquer une thérapie efficace aussitôt que possible pour éviter la détérioration de l’œdème cérébral post-brûlure. La deuxième phase (PBH 6-18), avec les caractéristiques pathologiques de l’œdème cytotoxique cérébral, s’est vérifiée pendant la période de l’apoptose. La troisième phase (PBH 18-24) était la période dangereuse de l’œdème cérébral. La pression intracrânienne augmentait rapidement à cause de la limitation de la cavité crânienne. En conséquence, la possibilité d’une hernie cérébrale était élevée. Une courbe à forme de S s’est produite dans le processus pathologique de l’œdème cérébral dans la phase précoce après la brûlure à la suite des grandes brûlures.
BIBLIOGRAPHY


G. WHITAKER INTERNATIONAL BURNS PRIZE – PALERMO (Italy)

Under the patronage of the Authorities of the Sicilian Region for 2007

By law n. 57 of June 14th 1983 the Sicilian Regional Assembly authorized the President of the Region to grant the “Giuseppe Whitaker Foundation”, a non-profit-making organisation under the patronage of the Accademia dei Lincei with seat in Palermo, a contribution for the establishment of the annual G. Whitaker International Burns Prize aimed at recognising the activity of the most qualified experts from all countries in the field of burns pathology and treatment.

Law n. 23 of December 2002 establishes that the prize becomes biannual. The next prize will be awarded in 2005 by the month of October in Palermo at the seat of the G. Whitaker Foundation.

The amount of the prize is fixed at Euro 20,660.00.

The Adjudicating Committee is composed of the President of the Foundation, the President of the Sicilian Region, the Representative of the National Lincei Academy within the G. Whitaker Foundation, the Dean of the Faculty of Medicine and Surgery of Palermo University or his nominee, a Representative of the Italian Society of Plastic Surgery, three experts in the field of prevention, pathology, therapy and functional recovery of burns, the winner of the prize awarded in the previous year and a legal expert nominated in agreement with the President of the Region as a guarantee of the respect for the scientific purpose which the legislators intended to achieve when establishing the prize.

Anyone who considers himself/herself to be qualified to compete for the award may send by January 31st 2007 his detailed curriculum vitae to: Michele Masellis M.D., Secretary-Member of the Scientific Committee, G. Whitaker Foundation, Via Dante 167, 90141 Palermo, Italy.

This paper was received on 9 August 2004.

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