Physiological thresholds for irreversible tissue damage in contusional regions following traumatic brain injury

A. S. Cunningham,1,2 R. Salvador,2 J. P. Coles,1,2 D. A. Chatfield,1,2 P. G. Bradley,1,2 A. J. Johnston,1,2 L. A. Steiner,1,2,4 T. D. Fryer,2 F. I. Aigbirhio,2 P. Smielewski,2 G. B. Williams,2 T. A. Carpenter,2 J. H. Gillard,2,4 J. D. Pickard2,3 and D. K. Menon1,2

1Division of Anaesthesia, Department of Medicine, 2Wolfson Brain Imaging Centre and 3Academic Neurosurgery Unit, Department of Clinical Neurosciences and 4Department of Radiology, University of Cambridge, Cambridge, UK

Correspondence to: Professor D. K. Menon, Division of Anaesthesia, University of Cambridge, Box 93, Addenbrooke’s Hospital, Cambridge CB2 2QQ.
E-mail: dkm13@wbic.cam.ac.uk

Cerebral ischaemia appears to be an important mechanism of secondary neuronal injury in traumatic brain injury (TBI) and is an important predictor of outcome. To date, the thresholds of cerebral blood flow (CBF) and cerebral oxygen utilization (CMRO₂) for irreversible tissue damage used in TBI studies have been adopted from experimental and clinical ischaemic stroke studies. Identification of irreversibly damaged tissue in the acute phase following TBI could have considerable therapeutic and prognostic implications. However, it is questionable whether stroke thresholds are applicable to TBI. Therefore, the aim of this study was to determine physiological thresholds for the development of irreversible tissue damage in contusional and pericontusional regions in TBI, and to determine the ability of such thresholds to accurately differentiate irreversibly damaged tissue. This study involved 14 patients with structural abnormalities on late-stage MRI, all of whom had been studied with ¹⁵O PET within 72 h of TBI. Lesion regions of interest (ROI) and non-lesion ROIs were constructed on late-stage MRIs and applied to co-registered PET maps of CBF, CMRO₂ and oxygen extraction fraction (OEF). From the entire population of voxels in non-lesion ROIs, we determined thresholds for the development of irreversible tissue damage as the lower limit of the 95% confidence interval for CBF, CMRO₂ and OEF. To test the ability of a physiological variable to differentiate lesion and non-lesion tissue, we constructed probability curves, demonstrating the ability of a physiological variable to predict lesion and non-lesion outcomes. The lower limits of the 95% confidence interval for CBF, CMRO₂ and OEF in non-lesion tissue were 15.0 ml/100 ml/min, 36.7 μmol/100 ml/min and 25.9% respectively. Voxels below these values were significantly more frequent in lesion tissue (all \( P < 0.005 \), Mann–Whitney U-test). However, a significant proportion of lesion voxels had values above these thresholds, so that definition of the full extent of irreversible tissue damage would not be possible based upon single physiological thresholds. We conclude that, in TBI, the threshold of CBF below which irreversible tissue damage consistently occurs differs from the classical CBF threshold for stroke (where similar methodology is used to define such thresholds). The CMRO₂ threshold is comparable to that reported in the stroke literature. At a voxel-based level, however (and in common with ischaemic stroke), the extent of irreversible tissue damage cannot be accurately predicted by early abnormalities of any single physiological variable.

Keywords: traumatic brain injury; CBF; cerebral metabolism; physiological thresholds; prediction

Abbreviations: CBF = cerebral blood flow; CMRO₂ = cerebral metabolic rate of oxygen utilization; FLAIR = fluid-attenuated inversion recovery; NPV = negative predictive value; OEF = oxygen extraction fraction; PPV = positive predictive value; ROI = region of interest; TBI = traumatic brain injury

Introduction

The principal aim in the management of patients with severe traumatic brain injury (TBI) is the prevention or amelioration of secondary neuronal injury. Cerebral ischaemia appears to be an important mechanism of secondary brain damage after TBI. Post-mortem studies have consistently demonstrated ischaemic damage (Graham and Adams, 1971; Graham et al., 1989), and recent antemortem studies have demonstrated regional ischaemia early after TBI, and shown that the volume of ischaemic tissue is related to neurological outcome (Coles et al., 2004).

In experimental and clinical stroke the concept of the ischaemic penumbra, tissue that although ischaemic, is still morphologically intact and potentially viable, has long been recognized (Astrup et al., 1981; Jones et al., 1981; Heiss et al., 1992). In traumatic brain injury there is increasing evidence for the existence of a ‘traumatic penumbra’ (Coles et al., 2002, 2004; Imberti et al., 2002; Marion et al., 2002; Meixensberger et al., 2003; Menon, 2003; Nordstrom et al., 2003)—tissue that is most at risk of secondary ischaemic neuronal injury and that will be most affected by changes in physiology or therapeutic interventions.

A number of studies in acute ischaemic stroke have correlated the morphological outcome of tissue with the acute physiology and determined physiological thresholds of cerebral blood flow (CBF) and cerebral oxygen utilization (CMRO₂) for the development of irreversible tissue damage (Baron et al., 1984; Powers et al., 1985; Marchal et al., 1999) and for the penumbra. Using such thresholds, investigators have attempted to acutely identify irreversibly damaged, penumbral and normal tissue (Furlan et al., 1996; Marchal et al., 1996; Heiss et al., 2001), and to monitor the efficacy of therapeutic strategies such as reperfusion (Heiss et al., 1998). In TBI, the early identification of irreversibly damaged and penumbral tissue could have considerable therapeutic and prognostic implications. Early identification of such tissue could offer the opportunity to better study the effects of neuroprotective interventions, surgery and drugs, and formulate strategies that best preserve vulnerable tissue from secondary injury in individual patients. In particular, it may be possible to predict neurological outcome and determine whether such strategies lead to significant clinical benefits with improved neurological outcome.

However, the applicability of thresholds for tissue damage derived from stroke studies to head-injured patients is questionable. The pathogenesis of acute ischaemic stroke is well described; macrovascular occlusion leads to perfusion limited ischaemia, usually resulting in a topographically well-defined ischaemic core and surrounding penumbra (Baron, 1999). In TBI the pathophysiological mechanisms responsible for neuronal death have not been fully elucidated. Previous studies in TBI have commonly used reduced perfusion to define ischaemia after TBI, and extrapolated classical stroke CBF thresholds for irreversible tissue damage. However, cerebral metabolism is commonly reduced following TBI (Glenn et al., 2003), due to the effects of trauma itself (Bergsneider et al., 2000; Verweij et al., 2000) and the concurrent use of sedative agents. Consequently, low CBF may result from preserved flow–metabolism coupling, and CBF thresholds for tissue survival might be expected to be lower than in stroke. Conversely, it is possible that excitotoxicity may lead to increases in cerebral metabolism that are not met by seemingly adequate CBF levels, and CBF thresholds for survival may be increased. Furthermore, the effects of trauma on cerebral physiology show considerable spatial (Bouma et al., 1992; Coles et al., 2002, 2004) and temporal heterogeneity (Martin et al., 1997), both between and within patients. It may not be possible to determine universal thresholds for tissue viability that can be applied to all patients or even across all regions in an individual patient.

Cerebral contusions provide a useful model to address the issues discussed above, since they are usually associated with structural abnormalities at follow-up imaging, allowing clear definition of lesion tissue. Further, the pericontusional region is recognized as a component of the traumatic penumbra (Menon, 2003; Nordstrom et al., 2003). PET imaging of patients with TBI provides a comprehensive characterization of physiological abnormalities in such regions. To date, no previous studies in TBI have correlated PET studies with eventual morphological outcome. The aim of this study was to determine, in the acute phase following TBI, physiological thresholds for the development of irreversible tissue damage in contusional and pericontusional tissue. To derive these thresholds we have used methodology similar to that used to derive stroke thresholds, and we compared these thresholds with classical stroke thresholds. Further, we aimed to determine if any such thresholds could accurately differentiate ultimately damaged tissue from undamaged tissue.

To address these aims we have co-registered acute stage PET images from patients with significant TBI with later structural MRIs, and investigated CBF, CMRO₂ and oxygen extraction fraction (OEF) in lesion and non-lesion brain tissue at a region of interest (ROI)-and voxel-based level. It is important to emphasize that, while selective neuronal loss is known to occur in the absence of structural imaging changes following head injury (Friedman et al., 1998), these analyses concentrated on structurally visible lesions.

Methods

Subjects

Patients were retrospectively selected from our database of patients with significant TBI [admission Glasgow Coma Scale (GCS) or subsequent deterioration in GCS to 8 or less] who had been admitted to Addenbrooke’s Hospital Neurosciences Critical Care Unit (NCCU). Patients were considered eligible for inclusion in this study if they fulfilled the following criteria: (i) they had been studied with PET within 72 h of injury; they had undergone follow-up MRI 3–18 months after injury; (iii) follow-up MRI demonstrated structural abnormality; and (iv) all images were technically adequate.

Assent was obtained from the next of kin for all patient studies during the acute phase after injury. Follow-up studies
were performed after informed consent had been obtained from patients or assent had been obtained from the next of kin, in accordance with the Declaration of Helsinki. All studies were approved by the Local Research Ethics Committee at Addenbrooke’s Hospital, and by the Administration of Radioactive Substances Advisory Committee of the United Kingdom.

Clinical protocols
All the patients were managed with protocol-driven therapy, as has been described previously (Menon, 1999) (see Appendix 1, available at Brain Online), aimed at maintaining intracranial pressure below 20 mmHg and cerebral perfusion pressure greater than 70 mmHg. All patients were intubated and mechanically ventilated, and sedated with propofol (2–5 mg/kg/h) and fentanyl (1–2 μg/kg/h). CPP was maintained with an infusion of norepinephrine and/or dopamine, adjusted as necessary. During all PET studies patients were paralysed with atracurium; sedative infusions were left unchanged and haemodynamic stability was ensured by titrating fluids and vasopressors. PaCO2 was measured at regular intervals and kept stable (at between 4.0 and 4.5 kPa) by adjusting ventilation as necessary.

PET studies
PET studies were performed within 72 h of patients’ admission to the NCCU, using a General Electric Advance scanner (GE Medical Systems, Milwaukee, WI, USA), as previously described (Coles et al., 2004). A 10-min transmission scan, using two rotating germanium-68 rods, was performed for all patients, and used to correct the emission data for photon attenuation. Emission data were acquired in 3D mode during a 20-min steady-state infusion of 800 MBq of H215O, following a 60-s inhalation of 300 MBq of C 15O, and in 2D mode during a 20-min steady-state inhalation of 7200 MBq of 15O2. Images were reconstructed using the PROMIS 3D filtered back-projection algorithm, with corrections applied for attenuation, scatter, randoms and dead time. From radioactivity concentrations in brain and arterial blood, parametric maps of CBF, CMRO2 and OEF were calculated, using previously described standard PET models (Phelps et al., 1979; Frackowiak et al., 1980; Herscovitch and Raichle, 1985; Lammertsma et al., 1987).

MRI studies
Follow-up late-stage MRI studies were performed 3–18 months after injury on a 3-tesla Bruker Medspec S300 (Bruker BioSpin MRI, Ettlingen, Germany). A number of sequences were obtained, including FLAIR image (fluid-attenuated inversion recovery), for co-registration purposes.

Image analysis
PET images were analysed using custom-designed automated software [PETAN (Smielewski et al., 2002)], which incorporates elements of several software packages, including Matlab 6.0 (MathWorks, Natick, MD, USA), statistical parametric mapping (SPM99; Wellcome Department of Cognitive Neurology, London, UK) and Analyze (AnalyzeDirect, Lenexa, KS, USA). All the PET images were individually co-registered with the late FLAIR MRI using vtkCISG software (Computational Imaging Sciences Group, Kings College, London; http://www.image-registration.com/), and were spatially smoothed using a 6 mm Gaussian filter.

Construction of regions of interest (ROIs)
We identified areas of structural abnormality on the MR FLAIR images that corresponded to traumatic contusions (mixed or high-density lesions) on initial acute CT. Without knowledge of the PET data, we then constructed lesion ROIs on the MR FLAIR images using the threshold tool in Analyze image analysis software to delineate areas of hypo- or hyperintensity. Each contusion was outlined on all relevant slices and considered as one ROI. Where possible, the contusions were then mirrored onto the contralateral hemisphere, using Analyze software, to produce non-lesion ROIs. Where bilateral contusions were present it was not possible to construct mirror non-lesion ROIs. All the ROIs from the MRIs were then projected onto the corresponding co-registered PET images (Fig. 1). Values for CBF, CMRO2, and OEF were determined for each ROI, and also for the entire population of voxels within the ROIs.

Statistical analysis, including examination of predictive ability of thresholds
ROI-based analysis
Statistical analysis was undertaken using Statview (Version 5, 1998; SAS Institute, Cary, NC, USA). Across each group of ROIs, values for each physiological variable were averaged to give overall values for lesion and non-lesion ROIs, and compared using the Mann–Whitney U-test. A P value <0.05 was considered significant. To ensure that the lesion ROIs that could be mirrored to produce non-lesion ROIs
were representative of the whole group of lesion ROIs, these were compared with those lesion ROIs that could not be mirrored. Descriptive statistics are given as median and interquartile range, unless otherwise stated.

**Voxel-based analysis**

**Determination of 95% confidence limits.** For the entire set of voxel values of CBF, CMRO₂ and OEF in non-lesion ROIs, we determined the upper and lower 95% confidence limits. For each PET physiological variable, we determined if the percentage of voxels in lesion tissue with values below these ‘thresholds’ was significantly greater than that in non-lesion tissue (Mann–Whitney U-test; P < 0.05 was considered significant).

**Determination of predictive ability of physiological variables.** A detailed description of the statistical methods used to determine if the physiological variables could predict tissue outcome is given in Appendix 2 (Appendix 2 can be viewed at Brain Online); the main steps are described below.

Using the complete set of voxel values of CBF, CMRO₂ and OEF, for all lesion and non-lesion ROIs derived from MRIs, we produced the cumulative probability function for voxels coming from lesion and non-lesion ROIs for each physiological variable. For any proposed threshold value of a physiological variable, these probability curves were used to directly determine the sensitivity of that threshold (i.e. the proportion of correctly classified voxels among truly lesion voxels); the specificity (the proportion of correctly classified voxels among truly non-lesion voxels); the false-negative rate; and the false-positive rate.

In the acute phase after TBI we only have the PET maps of CBF, CMRO₂ and OEF to predict tissue outcome. The ability of a proposed threshold to predict eventual tissue outcome in the acute period after TBI is given by the positive predictive value (PPV; the probability that a voxel classified as lesion by PET will truly be lesion) and negative predictive value (NPV; the probability that a voxel classified as non-lesion will truly be non-lesion). To determine these values, we must also account for the prior likelihood of a voxel being lesion or non-lesion. To obtain an estimate of this probability, we determined all these probabilities numerically at the lower 95% confidence limit values, and also at two further possible threshold values: the threshold corresponding to a 95% PPV, and the threshold corresponding to 95% sensitivity.

**Results**

**General clinical data**

Fourteen patients met the criteria for inclusion in the study; the demographic and clinical characteristics of these patients are shown in Table 1. As is typical of TBI, the majority of patients were young males (mean age 29 years, range 17–41 years). Patients had a mean (range) post-resuscitation Glasgow Coma Score of 8 (4–12). The mean (range) time to acute PET imaging was 43 (8–72) h and the mean (range) time to follow-up MRI was 239 (96–575) days after injury.

**Characteristics of ROIs studied**

Across the 14 eligible patients, a total of 31 lesion contusional ROIs were identified. One patient had four contusions, two had three contusions, three had one contusion and the remainder had two contusions. Three patients had surgery prior to the PET scans; two patients had removal of haematoma (in these patients the contusion ROIs represented anatomically distinct contusions that were not surgically evacuated), and one patient had removal of acute subdural haematoma and contusional tissue (but still had hyperintense lesion tissue on the follow-up MRI which was used as the lesion ROI). Twenty-one lesion ROIs could be mirrored to the contralateral hemisphere to produce 21 non-lesion ROIs. The lesion ROIs contained a total of 152 800 voxels; non-lesion ROIs contained a total of 108 527 voxels.

<p>| Table 1 Clinical and demographic characteristics of the fourteen patients |
|------------------|-----------------|----------------|----------------|---------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>GCS</th>
<th>Anatomical location of injury</th>
<th>Time to PET (h)</th>
<th>Time to MRI (days)</th>
<th>Marshall category</th>
<th>GOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24</td>
<td>M</td>
<td>9</td>
<td>Bilateral frontal</td>
<td>72</td>
<td>230</td>
<td>DI II</td>
<td>GR</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>M</td>
<td>12</td>
<td>Left frontal</td>
<td>15</td>
<td>296</td>
<td>NML</td>
<td>MD</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>M</td>
<td>9</td>
<td>Bilateral frontal and left temporal</td>
<td>8</td>
<td>138</td>
<td>NML</td>
<td>MD</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>M</td>
<td>9</td>
<td>Left frontotemporal</td>
<td>30</td>
<td>534</td>
<td>NML</td>
<td>GR</td>
</tr>
<tr>
<td>5</td>
<td>22</td>
<td>F</td>
<td>7</td>
<td>Left fronto-temporo-parietal</td>
<td>54</td>
<td>174</td>
<td>NML</td>
<td>SD</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>M</td>
<td>4</td>
<td>Right frontal</td>
<td>34</td>
<td>207</td>
<td>DI III</td>
<td>GR</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>M</td>
<td>7</td>
<td>Right frontal and left parieto-occipital</td>
<td>57</td>
<td>229</td>
<td>NML</td>
<td>SD</td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>M</td>
<td>8</td>
<td>Left frontotemporal</td>
<td>18</td>
<td>184</td>
<td>EML</td>
<td>GR</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>M</td>
<td>8</td>
<td>Right frontotemporal</td>
<td>60</td>
<td>140</td>
<td>EML</td>
<td>MD</td>
</tr>
<tr>
<td>10</td>
<td>41</td>
<td>M</td>
<td>12</td>
<td>Left temporal</td>
<td>57</td>
<td>177</td>
<td>DI III</td>
<td>GR</td>
</tr>
<tr>
<td>11</td>
<td>32</td>
<td>M</td>
<td>6</td>
<td>Bilateral fronto-temporo-parietal</td>
<td>72</td>
<td>96</td>
<td>EML</td>
<td>MD</td>
</tr>
<tr>
<td>12</td>
<td>19</td>
<td>M</td>
<td>5</td>
<td>Bilateral frontal</td>
<td>70</td>
<td>203</td>
<td>DI II</td>
<td>MD</td>
</tr>
<tr>
<td>13</td>
<td>23</td>
<td>M</td>
<td>4</td>
<td>Right frontoparietal</td>
<td>44</td>
<td>169</td>
<td>DI III</td>
<td>MD</td>
</tr>
<tr>
<td>14</td>
<td>31</td>
<td>M</td>
<td>11</td>
<td>Left frontal</td>
<td>16</td>
<td>575</td>
<td>NML</td>
<td>MD</td>
</tr>
</tbody>
</table>

Marshall category was recorded on admission imaging (Marshall et al., 1991). NML = non-evacuated mass lesion; EML = evacuated mass lesion; DI = diffuse injury; GCS = postresuscitation Glasgow Coma Score (Teasdale and Jennett, 1974); GOS = Glasgow Outcome Score (Jennett and Bond, 1975); GR = good recovery; MD = moderate disability; SD = severe disability; VS = persistent vegetative state; D = death.
Comparison of physiological variables for lesion and non-lesion ROIs

CBF, CMRO\textsubscript{2} or OEF and relevant statistics are summarized in Table 2 and Fig. 2. Statistical analysis demonstrated that lesion ROIs had significantly lower CBF, CMRO\textsubscript{2} and OEF values compared with non-lesion ROIs (all $P < 0.001$, Mann–Whitney $U$-test). There was no statistical difference between mirrored lesion ROIs and non-mirrored lesion ROIs.

95% confidence limits for lesion and non-lesion voxel populations

The CBF, CMRO\textsubscript{2} and OEF distributions from voxels in lesion ROIs were distinctly positively skewed and shifted to the left compared with non-lesion ROIs, although there was considerable overlap of the distributions (Fig. 3). Direct calculations of the 95% confidence limits for lesion and non-lesion voxel populations (using a method similar to that described by Marchal et al., 1999) are given in Table 3.

The lower 95% confidence limits for non-lesion voxels were 15.0 ml/100 ml/min, 36.7 $\mu$mol/100 ml/min and 25.9% for CBF, CMRO\textsubscript{2} and OEF respectively. Table 4 shows the proportion of voxels with values below these thresholds in lesion and non-lesion tissue; voxels below these values occurred significantly more frequently in lesion tissue when compared with non-lesion tissue. However, it is evident that there is considerable overlap of the two populations for all physiological variables, the upper 95% confidence limit for lesion voxels being much greater than the lower 95% confidence limit for non-lesion voxels.

Table 2 ROI-based comparison of physiological variables: median (IQR), $n = 14$

<table>
<thead>
<tr>
<th></th>
<th>CBF (ml/100 ml/min)</th>
<th>CMRO\textsubscript{2} ($\mu$mol/100 ml/min)</th>
<th>OEF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-lesion ROIs</td>
<td>33.0 (9.4)</td>
<td>76.4 (33.1)</td>
<td>36.4 (16.7)</td>
</tr>
<tr>
<td>Lesion ROIs</td>
<td>16.9 (12.5)</td>
<td>36.7 (32.1)</td>
<td>25.3 (13.2)</td>
</tr>
<tr>
<td>$P^*$</td>
<td>$&lt;0.005$</td>
<td>$&lt;0.005$</td>
<td>$&lt;0.005$</td>
</tr>
</tbody>
</table>

*Mann–Whitney $U$-test.

Fig. 2 Comparison of physiological variables for lesion and non-lesion ROIs. In the box and whisker plots the central line in each box denotes the median value, the lower and upper boundaries of the box denote interquartile range, and error bars denote the 10th and 90th centiles.

Fig. 3 (A) CBF histogram for all lesion and non-lesion voxels. (B) CMRO\textsubscript{2} histogram for all lesion and non-lesion voxels. (C) OEF frequency histogram for all lesion and non-lesion voxels.
Determination of probabilities and examination of thresholds

Lesion voxels comprised 4.3% of the entire supratentorial brain volume, giving estimates of prior probability of lesion and non-lesion outcome of 0.043 and 0.957.

Figure 4 shows the PPV and NPV plotted against the physiological variables. From Fig. 4 it can be seen that, while the NPV is high for almost all values of physiological variables (as would be expected since lesion voxels constitute only a small proportion of the brain volume), the PPV is high only at extremely low values of the physiological variables. Thus, it is only at these very low values of CBF, CMRO$_2$ or OEF that there is a high probability that a voxel classified as lesion by PET will truly become lesion. Table 5A gives the probability

![Graphs showing PPV and NPV against physiological variables](image)

**Table 3** Upper and lower 95% confidence limits (CL) for physiological variables in lesion and non-lesion voxel populations

<table>
<thead>
<tr>
<th></th>
<th>CBF (ml/100 ml/min)</th>
<th>CMRO$_2$ (µmol/100 ml/min)</th>
<th>OEF (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesion lower 95% CL</td>
<td>3.42</td>
<td>0.22</td>
<td>0.13</td>
</tr>
<tr>
<td>Lesion upper 95% CL</td>
<td>46.6</td>
<td>96.5</td>
<td>54.3</td>
</tr>
<tr>
<td>Non-lesion lower 95% CL</td>
<td>15.0</td>
<td>36.7</td>
<td>25.9</td>
</tr>
<tr>
<td>Non-lesion upper 95% CL</td>
<td>47.2</td>
<td>139.3</td>
<td>67.5</td>
</tr>
</tbody>
</table>

**Table 4** Proportion of voxels with values below non-lesion tissue 95% lower confidence limits for CBF, CMRO$_2$ and OEF

<table>
<thead>
<tr>
<th>PET variable</th>
<th>Non-lesion lower 95% CI</th>
<th>Lesion tissue voxels (%)</th>
<th>Non-lesion tissue voxels (%)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF</td>
<td>15.0 ml/100 ml/min</td>
<td>43.1</td>
<td>5.2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>CMRO$_2$</td>
<td>36.7 µmol/100 ml/min</td>
<td>52.1</td>
<td>5.0</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>OEF</td>
<td>25.9%</td>
<td>46.7</td>
<td>5.0</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

*Mann–Whitney U-test.
estimates associated with the 95% lower confidence limits. It is evident that these values perform poorly in separating lesion and non-lesion tissue.

At the physiological values at which there is a 95% PPV for lesion outcome, the sensitivity is very low (Table 5B); thus, a large proportion of lesion voxels are not identified by such thresholds and the extent of tissue damage is greatly underestimated. Alternatively, if we consider the physiological threshold at which the majority of lesion voxels were identified (i.e. at 95% sensitivity), then the PPVs of such thresholds are very low and a large proportion of non-lesion voxels are misclassified as lesion-based on PET values. The extent of irreversible tissue damage is therefore greatly overestimated (Table 5C).

In pericontusional regions the prior probability of lesion outcome would be much greater. Therefore, we examined the probability estimates associated with an a priori probability of lesion outcome of 0.5 (Table 6). Although the predictive ability of the physiological variables was improved, it remains the case that lesion tissue could not be accurately mapped, based upon univariate physiological thresholds.

Discussion

Thresholds for irreversible neuronal damage used in studies of TBI have usually been taken from the stroke literature. We have used PET to determine the lower limits of CBF, CMRO₂ and OEF associated with tissue survival in TBI. We have found that the CBF below which tissue is unlikely to be viable differs from the stroke CBF threshold (although the CMRO₂ threshold is comparable), a finding which underlines the problems of translating stroke CBF thresholds to TBI. Furthermore, we have investigated the ability of single physiological variables to predict tissue outcome, and have demonstrated that [in common with ischaemic stroke (Warach, 2001)] there is no absolute threshold of any single physiological variable that provides accurate differentiation of damaged and undamaged tissue.

Methodological issues

A number of methodological issues in this study must be addressed. Firstly, PET imaging (like xenon-CT), provides only a snapshot picture of the metabolic state of the brain. Physiological variables, in particular CBF, have been shown to vary with time following injury and the fate of tissue will depend on the duration as well as the extent of ischaemia (or other pathophysiological derangement); thus, thresholds for tissue viability would also be expected to vary with the time after injury and the duration of physiological derangement. We imaged all patients within 72 h of initial injury; however, it is possible that patients suffered differing durations of ischaemia that could not be accounted for by a single PET scan and that influenced our analysis, and that we may have obtained different results had it been possible to image patients at an earlier time point. A more accurate determination of tissue viability would take account of this time-dependence; however, such repeated or continuous measurement would be difficult to perform. A further problem is that secondary neuronal injuries following head
injury can occur a considerable time after the initial injury (Jones et al., 1994). It is therefore possible that some lesion areas on late MRIs may have resulted from later physiological insults, leading to inaccuracies when attempting to determine thresholds based on their earlier physiology.

Secondly, in attempting to determine thresholds for tissue viability, we used a voxel-based approach in which we included all parenchymal voxels in our analysis. Grey and white matter have differing physiological CBF and CMRO$_2$ (Marchal et al., 1992) and may have differing thresholds for neuronal survival (Marcoux et al., 1982; Pantoni et al., 1996), with ischaemia less well tolerated by grey compared with white matter. In theory, if the proportion of grey and white matter were different in lesion and non-lesion ROIs, then this would alter CBF, CMRO$_2$ and OEF values and could influence any determination of physiological thresholds. However, since we used mirrored lesion and non-lesion ROIs this is unlikely to have affected our results. Furthermore, even if there are different thresholds for tissue viability in grey and white matter after TBI, a method of segmenting brain tissue in the acute phase would be required for this to be clinically relevant; currently, given the lack of grey–white contrast in contusional and pericontusional tissue, any such segmentation based on structural or PET imaging is not possible.

We chose a voxel-based analysis of the data since this would allow subsequent objective mapping of irreversible tissue damage. Thresholds derived from ROI-based analysis would only be directly applicable to similar ROIs, and thus would not be appropriate for future mapping of tissue damage. However, voxel data are less robust than data from larger ROIs, due to increased partial volume error and a reduction in signal-to-noise characteristics. This may result in an increased spread of metabolic values.

Thirdly, we compared acute PET images with later MRIs. Acutely, contusions are usually swollen; however, over the months following injury there is often local (and global) cerebral atrophy and contraction of contusional areas. This clearly presents difficulties when correlating images obtained at different time points after injury. We have attempted to minimize the errors that could result from inaccurate comparisons between the images by using image co-registration (including algorithms for co-registration of grossly distorted images). While there are problems with co-registration, it is important to emphasize that the methodology we employed provides the best possible solution to a perennial problem in imaging research.

Finally, we must consider the statistical methods used to determine physiological thresholds for tissue damage following TBI, and to examine how well lesion and non-lesion tissue can be separated and identified by the physiological variables. No previous studies in TBI have attempted to determine thresholds for tissue survival. However, there are a number of studies in acute stroke that have derived thresholds for tissue viability (Baron, 1983; Powers et al., 1985; Furlan et al., 1996; Marchal et al., 1999; Heiss et al., 2001). Such studies have used differing methods in determining thresholds for tissue survival, but all are probability-based. A problem with any probabilistic approach, however, lies in deciding which values are the best estimates of thresholds for tissue damage and viability, since for most conditions there will be some overlap in the values of physiological variables in lesion and non-lesion regions. A number of previous stroke studies have used the lower range or lower 95% confidence limit of physiological values in normal or non-infarcted tissue as thresholds for irreversible tissue damage (Powers et al., 1985; Marchal et al., 1999); since infarcted tissue was significantly more likely to have CBF and CMRO$_2$ values below this, it was concluded that these values provide a reasonable estimate of the threshold at which tissue viability is compromised. We have applied similar methodology to TBI, to derive thresholds for irreversible tissue damage. Such analyses, even in the context of TBI, do lead to meaningful results regarding the values of physiological variables below which tissue survival is highly unlikely.

However, at the voxel-based level, we have demonstrated that there is a large degree of overlap of physiological variables in lesion and non-lesion tissue; in common with stroke studies, this limits the ability of these thresholds to reliably separate irreversibly damaged tissue. Thus, while tissue with CBF, CMRO$_2$ (or OEF) values below the derived thresholds is highly likely to ultimately become lesion tissue, much lesion tissue actually has values above the thresholds. The clinical usefulness of such thresholds is thus limited since they are poor at determining the extent of lesion tissue, and even if CBF and/or CMRO$_2$ could be increased to above these ‘thresholds’ it is uncertain whether tissue would survive. Similarly, it is not possible to identify thresholds that would include most lesion tissue in the acute estimation of the extent of irreversibly damaged tissue without including a substantial amount of non-lesion tissue.

We used Bayesian statistical analysis in our voxel-based approach to predicting tissue outcome. Lesion voxels comprised only a relatively small proportion of the whole brain volume, leading to a low prior probability of lesion outcome. However, questions regarding tissue fate are usually asked in the context of clinical knowledge, and the a priori probabilities of voxel fate may be quite different when a similar analysis is applied, for example, to pericontusional tissue, which may have a high risk of incorporation into an expanding lesion. Thus we examined the predictive accuracy of the derived thresholds with a priori probability of lesion outcome set to 50% (Table 6). Under such circumstances, lesion and non-lesion tissue was better differentiated on the basis of the acute physiology; however, there were still no thresholds that would accurately predict tissue outcome.

**Comparison with previous studies and interpretation of results**

As stated earlier, no previous studies have co-registered acute PET images to later structural images and sought thresholds for tissue viability in TBI. However, we can compare our
ROI-based analysis of the acute physiology of ultimately lesion and non-lesion areas with previous studies that have investigated the physiological derangements in and around traumatic cerebral contusions. In this study the averaged CBF in areas that eventually progressed to structural abnormality was significantly reduced (median CBF 16.9 ml/100 ml/min) compared with non-lesion areas. This is in keeping with previous studies of CBF in and around contusions (Schröder et al., 1995; McLaughlin and Marion, 1996; von Oettingen et al., 2002; Furuya et al., 2003; Steiner et al., 2003), which, although reporting a wide range of flows, have generally concurred in finding average CBF less than 20 ml/100 ml/min (Schröder et al., 1995; von Oettingen et al., 2002; Furuya et al., 2003; Steiner et al., 2003). Similarly, our finding that CMRO₂ was reduced both within ultimately lesion and non-lesion areas is in agreement with previous studies that have established that CMRO₂ is decreased both within focal contusions and within normal-appearing brain (Tenjin et al., 1990; Hutchinson et al., 2002; Glenn et al., 2003). There is less literature regarding OEF values within cerebral contusions, since this requires PET for accurate determination. A recent voxel-based study of the entire brain parenchyma has demonstrated the presence of distributed ischaemia within 24 h of TBI, as defined by an increased OEF (Coles et al., 2004). In contrast, previous studies have found low ROI and global OEF values after TBI, suggesting that global ischaemia is uncommon (Diringer et al., 2000, 2002). Similarly, we found reduced OEF in ultimately non-lesion ROIs and a markedly reduced OEF in lesion ROIs. These differences may reflect difficulties in demonstrating ischaemia at differing time points following injury, when early compensated ischaemia will be associated with a high OEF, while decompensation and progressive cell death within the voxel will result in progressive reductions in OEF, culminating in very low values in voxels where most neurons have died. These variations are also further confounded by spatial averaging of OEF within structurally defined regions or across the whole brain, possibly due to the dilution of small regions with increased OEF by larger regions with normal or low OEF. It is also possible that there are mechanisms of ischaemic cell death that are not characterized by increases in OEF, such as microvascular ischaemia (Menon et al., 2004) and mitochondrial dysfunction (Verweij et al., 2000; Clausen et al., 2001).

Studies of TBI have commonly used a threshold taken from the stroke literature, so it is of relevance to compare our results with these previous stroke studies. Classic PET studies in stroke patients determined thresholds for irreversible tissue damage for CBF and CMRO₂ as 12 ml/100 ml/min and 65 μmol/100 ml/min (1.4 ml/100 ml/min) respectively, with a penumbral CBF threshold of around 20 ml/100 ml/min. These thresholds, in particular the at-risk CBF threshold of 20 ml/100 ml/min, have been used in a number of studies of TBI to define viable and non-viable tissue. However, these early studies were hampered by methodological problems, including the poor spatial resolution of early PET, and lack of image co-registration. More recent stroke studies have reported lower thresholds for irreversible tissue damage of between 5 and 8.5 ml/100 ml/min for CBF, and between 35 and 40 μmol/100 ml/min (0.8–0.9 ml/100 ml/min) for CMRO₂ (Furlan et al., 1996; Marchal et al., 1999; Heiss et al., 2001). In comparison, in TBI, the CBF threshold we report for irreversible damage (15.0 ml/100 ml/min) is higher than that of these more recent stroke studies (but lower than the commonly quoted threshold of 20 ml/100 ml/min), while the CMRO₂ threshold (36.7 μmol/100 ml/min) is very close to that found in these more recent studies.

Although it is possible to estimate physiological thresholds below which tissue is almost certainly destined for irreversible morphological abnormality, we have demonstrated that such thresholds cannot be used to accurately predict the full extent of irreversibly damaged tissue following TBI. Again, it is notable that more recent reviews addressing ischaemic stroke have suggested that tissue viability cannot be predicted based upon simple absolute physiological thresholds. There are a number of explanations that warrant consideration when considering why the physiological variables performed poorly in identifying irreversibly damaged tissue following TBI.

As discussed previously, both the extent and duration of ischaemia contribute to the evolution of irreversible tissue damage. This time dependence may mean that it is not possible to predict tissue outcome based upon measurement of CBF, CMRO₂ or OEF at a single time point after injury. It is also likely that tissue survival will be better predicted by complex interactions of physiological variables rather than simple univariate thresholds. Initial multivariate analysis appears to provide better separation of damaged and non-lesion tissue (Fig. 5), and further investigation may involve using multivariate statistical analysis or neural network techniques to determine if there are combinations of physiological

![Fig. 5 Three-dimensional plot of voxel physiological values, illustrating how multivariate analysis of PET variables may better separate lesion from non-lesion tissue.](image-url)
variables that better predict tissue outcome. Indeed techniques using multivariate algorithms (using data from acute perfusion-weighted and diffusion-weighted MRI scans), to generate voxel-based maps predicting tissue outcome, have been used with some success in stroke studies (Jiang et al., 1997; Wu et al., 2001).

In defining lesion ROIs, we delineated areas of pan-necrosis on the follow-up MRI scans. However, it is vitally important to emphasize that there may also be selective neuronal loss following TBI, which is not detected by conventional MRI. The effects of trauma on the cerebral circulation and cerebral metabolism are spatially variant and recent PET and microdialysis studies early after TBI have demonstrated distributed ischaemia, with significant ischaemia in apparently normal areas of brain (Marion et al., 2002; Nordstrom et al., 2003; Coles et al., 2004). Further evidence of selective neuronal loss is provided by 1H MR spectroscopy studies which have demonstrated reductions in N-acetyl aspartate, a marker of neuronal viability, in some apparently structurally normal areas of the brain (Condon et al., 1998; Garnett et al., 2000, 2001). Such selective neuronal loss may contribute to the large spread of physiological values that we observed both in lesion and in non-lesion tissue, as defined on the FLAIR images. In order to fully investigate differences in physiology in lesion and non-lesion areas, and possibly determine methods that separate them, it may be necessary to use follow-up techniques that detect selective neuronal loss, such as MR spectroscopy or 11C-flumazenil PET (Sette et al., 1993; Heiss et al., 2000).

The pathophysiology of TBI appears to be complex. Recent studies suggest that several pathophysiological mechanisms in addition to macrovascular perfusion-limited ischaemia may contribute to secondary neuronal loss, including mitochondrial dysfunction (Verweij et al., 2000; Clausen et al., 2001) and microvascular diffusion-limited ischaemia (Menon et al., 2004). Abnormalities in acute physiology may thus reflect the combined effects of several different pathophysiological processes, and heterogeneity in the effects of TBI, both within and between patients. Furthermore, there may be differences between patients in their susceptibility to the pathophysiological changes that follow TBI. Variations in the pathophysiology and distribution of damaged and at-risk tissue, and differences between patients, may mean that there are no clear physiological thresholds for tissue viability that can be universally applied in TBI.

Clinical implications

Our findings have implications for the future investigation and management of patients with TBI and for the interpretation of earlier studies in TBI. An important step in protecting cerebral tissue from secondary injuries following TBI would be the identification, in the acute phase, of tissue that is irreversibly damaged, and undamaged but at-risk tissue, ‘the traumatic penumbra’. If tissue outcome could be accurately predicted in the acute phase after injury, this could provide us with a surrogate marker to assess the effects of neuroprotective therapies, as well as an opportunity to better predict neuropsychological outcomes. A number of previous studies in TBI have used thresholds extrapolated from stroke studies, in particular CBF thresholds, to define irreversibly damaged tissue. Based on our findings, it is evident that stroke CBF thresholds cannot be translated to patients with TBI. Indeed, while the thresholds we report provide important estimates in TBI of the physiological values associated with irreversible damage, it is important to emphasize that, as with stroke, none of the physiological variables alone can be used to define acutely the extent of irreversibly damaged tissue. The identification of irreversibly damaged tissue, in the acute phase following TBI, may require more sensitive methods that detect distributed neuronal damage. These considerations may be particularly relevant when we move away from the localized contusional model of clinical head injury addressed in this paper, and attempt to find relevant physiological thresholds for diffuse ischaemic damage. Future attempts to produce predictive maps for tissue outcome may require using combinations of the physiological variables, and may need to account for time and patient variables.

Acknowledgements

These studies were supported by the Medical Research Council, a Technology Foresight Grant from the UK government, and by a Royal College of Anaesthetists/British Journal of Anaesthesia project grant. J.P.C. is funded by an Academy of Medical Sciences/Health Foundation Clinician Scientist award.

References


