Brain Research 1646 (2016) 334–341

Contents lists available at ScienceDirect

Brain Research

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

Research report

Cerebral and extracerebral vulnerability to hypoxic insults after diffuse traumatic brain injury in rats

Ségolène Mrozek [a,b,*], Aymeric Luzi [a,b], Leslie Gonzalez [a,b], Lionel Kerhuel [a,b], Olivier Fourcade [a,b], Thomas Geeraerts [a,b]

[a] Equipe d'accueil' Modélisation de l'aggression tissulaire et nociceptive', University Toulouse 3 Paul Sabatier, Toulouse, France
[b] Departement of Anesthesiology and Critical Care, University Hospital of Toulouse, Toulouse, France

ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form
3 June 2016
Accepted 4 June 2016
Available online 11 June 2016

Keywords:
Hypoxia-hypotension
Posttraumatic vulnerability
Brain-organ crosstalk
Tissue hypoxia

ABSTRACT

The post-traumatic brain vulnerability suggests that after traumatic brain injury (TBI), the brain may be more susceptible to posttraumatic hypoxic insults. This concept could be extended to 'peripheral' organs, as non-neurologic organ failure is common after TBI. This study aims to characterize and quantify cerebral and extracerebral tissue hypoxia with pimonidazole resulting from a standardized hypoxia-hypotension (HH) phase occurring after a diffuse experimental TBI in rats. Rats were allocated to Sham (n=5), TBI (n=7), HH (n=7) and TBI+HH (n=7) groups. Then, pimonidazole was injected and brain, liver, heart and kidneys were analysed. In the cerebral cortex, post-treatment hypoxia was higher in TBI+HH group than Sham group (p=0.003), HH group (p=0.003) and TBI group (p=0.002). Large trends in thalamus, hippocampus and striatum for the TBI+HH group compared to the other groups were observed. For the heart and liver, the 4 groups were comparable. For the kidneys, post-treatment hypoxia was higher in the TBI group compared to the Sham and HH groups, but not more than TBI+HH group. This study reveals that a posttraumatic hypoxic insult occurring after a severe TBI has major hypoxic consequences on brain structures. However, TBI by itself appears to induce renal hypoxia that is not enhanced by posttraumatic hypoxic insult.

1. Introduction

It is now well recognized that posttraumatic insults, such as hypoxia (Hemoglobin oxygen saturation: $SpO_2 < 90\%$ or arterial partial pressure in oxygen: $PaO_2 < 60$ mm Hg) and hypotension (systolic arterial pressure < 90 mm Hg), increase mortality and worsen neurological outcome in patients with severe traumatic brain injury (TBI) (Chesnut et al., 1993; Jeremitsky et al., 2003). The first posttraumatic hours are critical, and pre-hospital hypoxia and/or hypotension in severe TBI lead to an increase in mortality of 50% (Chesnut et al., 1993). The concept of posttraumatic brain vulnerability suggests that after TBI, the brain may be more susceptible to posttraumatic hypoxic insults. Animal TBI models allow the exploration of the pathophysiology of posttraumatic insults repercussion on the brain. Marmarou and Foda developed a standardised and reproducible impact-acceleration rat model of TBI to obtain diffuse axonal injuries with edema formation in the absence of parenchymal hemorrhage, avoiding focal injuries (Foda and Marmarou, 1994). The association of TBI with hypoxia and hypotension generates an increase in cerebral edema, mainly cytotoxic edema with the rise in ICP (Beaumont et al., 2002; Ishige et al., 1987a; Ito et al., 1996; Marmarou et al., 1994). Cerebral injuries related to posttraumatic insults are worsened in cases of pre-existing TBI (Geeraerts et al., 2008). Pathophysiology of secondary brain damages and cerebral vulnerability is still unclear but cerebral ischemia appears to be a major consequence and an important predictor of outcome (Coles et al., 2004). A study with positron emission tomography (PET) reported a high incidence of cerebral ischemia within the first days after severe TBI in humans (Coles et al., 2004). Some authors have mentioned depletion in high-energy phosphate metabolism, modifications in cell energy metabolism and impairment of oxidative metabolism with mitochondrial dysfunction to be a key point in cerebral vulnerability to hypoxia (Calabresi et al., 2003; Geeraerts et al., 2006; Geeraerts et al., 2008; Ishige et al., 1987b; Signoretti et al., 2001; Tavazzi et al., 2005). However, there is lack of experimental studies aimed

Abbreviations: ADC, apparent diffusion coefficient; AKI, acute kidney injury; DWI, diffusion-weighted imaging; HH, hypoxia/hypotension; MAP, mean arterial pressure; TBI, traumatic brain injury

* Correspondence to: Pôle Anesthésie Réanimation, Hôpital Pierre Paul Riquet, 31059 Toulouse cedex 9, France.
E-mail addresses: mrozek.s@chu-toulouse.fr (S. Mrozek), aymericluzi@yahoo.com (A. Luzi), gonzalez.l@chu-toulouse.fr (L. Gonzalez), lionel.kerhuel@gmail.com (L. Kerhuel), fourcade.o@chu-toulouse.fr (O. Fourcade), geeraerts.t@chu-toulouse.fr (T. Geeraerts).

http://dx.doi.org/10.1016/j.brainres.2016.06.007
0006-8993/

at characterizing and quantifying brain hypoxia after standardized TBI and posttraumatic insult.

For several years, the detrimental effects of TBI have also been described in non-neurological systems. Non-neurologic organ failure, such as acute kidney injury or acute lung injury, is common after TBI and is associated with worse outcomes (Zygun et al., 2005). Fifteen to 30% of patients with TBI present with heart failure, according to different studies (Huttemann et al., 2002; Zygun et al., 2005). Respiratory failure seems to be the most common type of non-neurologic organ dysfunction (Mazzeo et al., 2013; Zygun et al., 2005). Moreover, a higher incidence of acute kidney injury has been reported in TBI patients, with an increase in mortality and worse outcomes (Li et al., 2011). Some studies report that 20% of severe TBI mortality is due to multiple organ failure and not solely to cerebral injuries (Liu et al., 2008). TBI leads to immunodepression, inflammation in peripheral organs and an acute-phase response (Catania et al., 2009). The concept of post-traumatic vulnerability after TBI could therefore be extended to 'peripheral' organs, and not restricted to the brain. Indeed, for example, cardiovascular system seems to be more susceptible to hemorrhage and hypovolemia in case of high intracranial pressure (ICP) compared to healthy-brain condition. A blood loss corresponding to 25% of the blood volume, which is normally well-tolerated, could lead to genuine shock in cases of raised ICP (Kirkeby et al., 1995).

Brain-organ crosstalk could therefore lead to a complex interaction, resulting in a worsening of brain and 'peripheral' injuries. However, the incidence of systemic ischemic injuries after TBI and posttraumatic hypoxic insults has never been studied. This study aims to characterize and quantify cerebral and extracerebral tissue hypoxia resulting from a standardized hypoxia-hypotension phase occurring after diffuse experimental TBI in rats. Development of 2-nitroimidazole hypoxia markers (Hypoxyprobe-1) allows for the detection of the distribution of tissue hypoxia ($PO_2 < 10$ mm Hg) using the immunohistochemical technique (Ghafar et al., 2002). It has been validated for the assessment of the oxygenation of different tumors (Bache et al., 2008; Dubois et al., 2004; Hausermans et al., 2000; Raleigh et al., 2001) and the detection of hypoxia in normal tissue (Liu et al., 2009; Roberts et al., 1986; Westbury et al., 2007). Pimonidazole allows one to detect hypoxic but viable areas, where cellular oxygen pressure is ≤ 10 mm Hg, as it is reduced and binds to macromolecules in viable hypoxic cells (Ljungkvist et al., 2007). Recently, Huang et al. isolated areas of the cortex suffering from hypoxic damage in a rat model of cortical contusion induced by weight-dropping trauma with pimonidazole (Huang et al., 2010). Our hypothesis was that not only the brain but also peripheral organs, such as the heart, liver and kidneys, could be more susceptible to hypoxia in the acute phase of severe TBI.

2. Results

In total, 42 rats were studied but 16 (38%) were excluded for final analysis because of early death following refractory shock, cardiopulmonary arrest during the HH phase or accidental embolism (Table 1 for causes of exclusion). Mortality rate of TBI+HH rats was 46%, a rate that has been previously reported for this model (Geeraerts et al., 2006, 2008; Marmarou et al., 1994). Thereby, 26 rats were retained, allocated randomly beforehand to each four groups: Sham (n=5), TBI (n=7), HH (n=7), TBI+HH (n=7).

2.1. Physiologic characteristics and hemodynamic parameters

Animal's weights (mean weight: 463 ± 6 g) did not differ between the 4 groups: Sham, TBI, HH and TBI+HH (p=0.44). Rectal temperature was similar at T0 for each group (p=0.75). At 45 min, the TBI group presented a higher temperature than HH and TBI+HH groups (37.4 ± 0.2 °C vs. 36.5 ± 0.1 °C and 36.5 ± 0.2 °C; p=0.006 and p=0.014 respectively). Just before sacrifice, at 135 min, the rectal temperature was significantly different between Sham group and TBI group (36.8 ± 0.2 °C vs. 37.8 ± 0.2 °C, p=0.002). The volume of blood depletion during the HH phase was not significantly different between HH and TBI+HH groups (7.7 ± 1.0 ml vs. 9.2 ± 1.0 ml, p=0.15).

For hemodynamic parameters, at T0, MAP was not significantly different between the 4 groups (p=0.19) (Fig. 1). At 45 min, corresponding to the end of HH phase just before blood reinjection in groups subjected to HH, MAP was not different between HH and TBI+HH groups (35 ± 1 mm Hg vs. 38 ± 0.7 mm Hg, p=0.76) and between sham and TBI groups (115 ± 7 mm Hg vs. 110 ± 10 mm Hg, p=0.54). After HH phase, groups subjected to TBI (TBI and TBI+HH) had significant lower MAP than Sham and HH groups over time (HH group vs Sham group, TBI group and TBI+HH group, respectively p=0.89, p=0.004 and p=0.003; Sham group vs. TBI group and TBI+HH group, respectively p=0.002 and p=0.002; TBI group vs. TBI+HH group, p=0.87).

Table 1
Causes for exclusion in each group. TBI: *traumatic brain injury*. HH: *hypoxia-hypotension*. CPA: *cardiopulmonary arrest*.

	Sham group	TBI group	HH group	TBI+HH group
Early death:				
Refractory shock	1	3	1	1
CPA per HH	–	–	2	4
Embolism	0	1	1	1
Skull fracture	–	1	–	0
Total	1/6 (17%)	5/12 (42%)	4/11 (36%)	6/13 (46%)

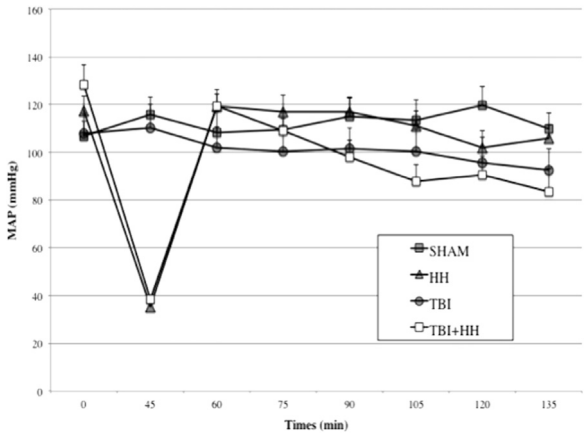

Fig. 1. Mean arterial pression (MAP) during procedure in each group. Values are means ± SEM. TBI: traumatic brain injury. HH: hypoxia-hypotension.

Table 2
Arterial blood gas samples at 140 min for each group. TBI: traumatic brain injury. HH: hypoxia-hypotension. PaO_2: arterial oxygen partial pressure. $PaCO_2$: arterial carbon dioxide partial pressure. NS: Not significant.

	Sham group	TBI group	HH group	TBI+HH group	p
PaO_2 (mm Hg)	212.5 ± 25.5	316 ± 30.4	321.3 ± 1.9	349.5 ± 43.8	*NS*
$PaCO_2$ (mm Hg)	50.8 ± 0.2	54.8 ± 0.8	57.0 ± 11.6	58.7 ± 16.8	*NS*
pH	7.35 ± 0.02	7.32 ± 0.03	7.31 ± 0.15	7.22 ± 0.10	*NS*
Lactates (mmol/L)	2.7 ± 0.2	1.9 ± 9.3	1.9 ± 0.2	2.8 ± 0.3	*NS*
Hemoglobin (g/dL)	15.0 ± 0.2	14.4 ± 0.2	15.6 ± 0.2	15.3 ± 0.5	*NS*