

[0017] FIG. 3a is a power blot template of MCAo (A) brain tissue 24 hours post-injury;

[0018] FIG. 3b is a power blot template of the sham (B) brain tissue 24 hours post-injury;

[0019] FIGS. 4a-4c: are diagrams showing comparison of the number of proteins exhibiting changes in abundance level from brain tissue of PBBI and MCAo injured rats as indicated by high throughput immunoblotting (HTPI);

[0020] FIG. 5a is a digital image showing Western Blot analysis (WB) of changes for selected proteins in both blood (plasma) and brain;

[0021] FIG. 5b is a graph showing quantitative measurement of WB density in brain tissues;

[0022] FIG. 6 is a diagram showing relevance of changes in expression of each of the five confirmed proteins detectable in blood of PBBI or MCAo injured rats;

[0023] FIG. 7 is a digital image of Western Blotting results for p43/EMAP-II protein expression in brain tissues at different time points following MCAo/PBBI;

[0024] FIG. 8 is a digital image of Western Blotting results for p43/EMAP-II protein expression in different types of tissue;

[0025] FIG. 9 is a conceptual drawing of a rat brain showing MCAo procedure;

[0026] FIG. 10 is a conceptual drawing of a rat brain showing PBBI procedure.

DETAILED DESCRIPTION

[0027] In the current study, the inventors applied high-throughput immunoblotting technology (HTPI; BD Power-Blot™) (Liu et al., 2006a) to study large scale differential protein patterns in rat brains 24 hours after either a penetrating ballistic-like brain injury (PBBI) (Williams et al., 2005; Williams et al. 2006a; Williams et al. 2006b) or middle cerebral artery occlusion (MCAo) (Tortella et al., 1999). Specifically, the inventors focused on identifying proteins with measurable changes in protein abundance following acute brain injury. They further hypothesized that a subset of those proteins would cross the BBB to be detected in blood. The value of this approach was demonstrated when five proteins (STAT3, Tau, PKA_{RTP}, 14-3-3Hand p43/EMAP-II), identified by our HTPI assay, were immunodetected as proteins released differentially into blood of PBBI or MCAo injured rats. It was found that the p43/EMAP-II candidate biomarker proved highly sensitive to detecting and distinguishing types and severities of brain injury and the underlying pathological processes, thereby advancing clinical diagnostics for evaluation of acute brain injury and patient monitoring.

[0028] EMAP-II is an inflammatory cytokine. Its pro-EMAP-II precursor is identical to the auxiliary p43 component of the aminoacyl-tRNA synthetase complex. EMAP-II domain of p43 is released readily from the complex after *in vitro* digestion with caspase 7 and is able to induce migration of human mononuclear phagocytes. P43 compares well with a molecular fuse that triggers the irreversible cell growth/cell death transition induced under apoptotic conditions. EMAP cytokine is released from the mammalian multisynthetase complex after cleavage of its p43/proEMAP-II component.

Materials and Methods

[0029] Adult male Sprague-Dawley rats (250-300 g; Charles River Labs, Raleigh, Va.) were used for all studies. Anesthesia was induced during all surgical manipulations by

5% isoflurane, and maintained at 2% isoflurane delivered in oxygen. All procedures were approved by the Walter Reed Army Institute of Research Animal Care and Use Committee. Research was conducted in compliance with the US Animal Welfare Act, *Guide for the Care and Use of Laboratory Animals* (National Research Council) and other federal statutes and regulations relating to animals and experiments involving animals. Animals were housed individually under a 12 hour light/dark cycle in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Middle Cerebral Artery Occlusion (MCAo) Model (Ischemic Injury)

[0030] Transient MCAo was carried out using the intraluminal filament model as described previously (Tortella et al., 1999). Briefly, the right external carotid artery was exposed, and its branches were coagulated. As shown in FIG. 9, a 3-0 uncoated monofilament nylon suture with rounded tip was inserted into the external carotid artery ECA, 2 and advanced until it lodged in the proximal region of the anterior cerebral artery, thus occluding the origin of the MCA, 1. The endovascular suture remained in place for 2 hours and was then retracted to allow reperfusion of blood to the middle cerebral artery (MCA). Sham (control) animals underwent the same surgical procedure but without the introduction of the filament into the MCA.

Penetrating Ballistic-Like Brain Injury (PBBI) Model

[0031] PBBI was produced by insertion of a specially designed probe, which connected to the Dragonfly Variable Pressure Waveform Generator (model HPD-1700; Dragonfly Inc., WV) to induce a rapid pressure pulse for expansion/contraction of the balloon inside the right hemisphere of brain (Moshang et al., 2003; Williams et al., 2005; Williams et al., 2006a; Williams et al., 2006b). Briefly, the balloon inflation device was constructed from a 20-gauge stainless steel tube with spatially fixed perforations at one end that were sealed by an airtight section of elastic tubing (PBBI balloon). The perforations are arranged in a pattern such that an air pulse delivered to an air cylinder will inflate the balloon in an elliptical shape. A "T" type junction, high-pressure manifold allows transmission of the pressure wave from the pressurizing piston and cylinder to both the implanted PBBI probe and the pressure transducer simultaneously. The induced pressure wave was measured directly by a precisely calibrated pressure transducer through a charge amplifier/coupler to a digital oscilloscope. The PBBI balloon does not expand until the pressure cylinder reaches near peak pressure with an average 'open' time of less than 10 ms as estimated by videotaping the balloon expansion event. Sham (control) animals underwent craniectomy without insertion of the PBBI probe. The rapid inflation and deflation of the balloon simulated a ballistic pressure wave causing a temporary intracranial cavity. Sham animals underwent craniotomy alone without insertion of the PBBI probe. In FIG. 10, the probe 4 and balloon expansion 3 are shown.

Brain Tissue Collection

[0032] Brain tissue was collected 24 hours following injury. Four experimental groups were assessed: PBBI, PBBI-sham, MCAo, and MCAo-sham. All animals were deeply anesthetized with ketamine/xylazine (70 and 6 mg/kg,