# Fresh Frozen Plasma Modulates Brain Gene Expression in a Swine Model of Traumatic Brain Injury and Shock: A Network Analysis

Martin Sillesen, MD, PhD, Ted Bambakidis, MSc, Simone E Dekker, MD, Yongqing Li, MD, PhD, Hasan B Alam, MD, FACS

**BACKGROUND:** Resuscitation with fresh frozen plasma (FFP) decreases brain lesion size and swelling in a swine model of traumatic brain injury and hemorrhagic shock. We hypothesized that brain gene expression profiles after traumatic brain injury and hemorrhagic shock would be modulated by FFP resuscitation.

**STUDY DESIGN:** Fifteen swine underwent a protocol of traumatic brain injury and hemorrhagic shock and 2 hours of shock followed by resuscitation with FFP, normal saline, or hetastarch (5/group). After 6 hours, brain RNA was isolated and hybridized onto a porcine gene ST 1.1 microarray. Weighted gene correlation network analysis was used to identify clusters of highly coexpressed genes. Principal component analysis identified cluster eigenvectors, indicating overall direction and magnitude of cluster gene expression. Using linear regression, cluster eigenvectors were associated with treatment as well as brain lesion size and swelling. Results were post-hoc corrected using false discovery rate. Relevant gene clusters were subjected to pathway analysis using the Reactome tool.

**RESULTS:** Network analysis identified 322 gene expression clusters (total of 12,462 coexpressed genes). Fresh frozen plasma resuscitation (but not normal saline or hetastarch) was positively associated with 2 distinct gene clusters (termed *A* and *B*) comprising 493 genes. Gene expression in both clusters was negatively associated with brain swelling, and cluster B was also negatively associated with lesion size. Pathway analysis revealed an upregulation of genes involved in metabolic and platelet signaling, as well as collagen formation and downregulation of inflammation.

**CONCLUSIONS:** Fresh frozen plasma resuscitation in this model was associated with downregulation of inflammatory pathway genes and expression of gene clusters mapping to increased metabolic and platelet signaling, which, in turn, was reversely associated with brain swelling. (J Am Coll Surg 2017;224:49–58. © 2016 by the American College of Surgeons. Published by Elsevier Inc. All rights reserved.)

**Disclosure Information: Nothing to disclose.**

Support: The study was funded by a grant to Dr Alam from the US Army Medical Research Material Command (T00521959).

Abstract presented at the American College of Surgeons 101st Annual Clinical Congress, Scientific Forum, Chicago, IL, October 2015.

Received August 20, 2016; Revised September 7, 2016; Accepted September 20, 2016.

From the Department of Surgical Gastroenterology (Sillesen) and Institute for Inflammation Research (Sillesen), Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark, and Department of Surgery, University of Michigan, Ann Arbor, MI (Bambakidis, Dekker, Li, Alam).

Correspondence address: Hasan B Alam, MD, FACS, Department of Surgery, University of Michigan Health System, 2920 Taubman Center/5331, 1500 E Medical Center Dr, Ann Arbor, MI 48109-5331. email: alamh@med.umich.edu

Trauma, including traumatic brain injury (TBI) and hemorrhagic shock (HS), is well known to induce clinically relevant perturbations in gene expression patterns of multiple cell lines.[1-3] Preclinical studies have indicated that these patterns can be further modulated by the choice of resuscitation strategy, emphasizing the important clinical role of gene expression after injury.[4] Although previous studies have focused on the detrimental effects of crystalloid resuscitation on both outcomes[5] and gene expression[4] after trauma, little is known about the effects of blood products on gene transcription after trauma, TBI, and HS. Balanced





http://dx.doi.org/10.1016/j.jamcollsurg.2016.09.015
ISSN 1072-7515/16

**Abbreviations and Acronyms**

BBB = blood-brain barrier
CO = cardiac output
FDR = false discovery rate
FFP = fresh frozen plasma
GS = gene significance
HS = hemorrhagic shock
ICP = intracranial pressure
MAP = mean arterial pressure
MM = module membership
TBI = traumatic brain injury

resuscitation strategies using high ratios of fresh frozen plasma (FFP) to packed RBCs form the mainstay of current trauma resuscitation paradigms, with multiple studies indicating a survival benefit of this regimen in trauma patients with critical bleeding.[6-8] Although less well studied, this effect might extend to patients with TBI,[9] as suggested by preclinical studies.[10,11]

Although the effect of FFP on coagulation derangements after trauma is usually perceived as the main mechanism of action, multiple preclinical studies have suggested that FFP exerts its protective effects through multiple alternate pathways. Studies have suggested a protective effect on the endothelium,[12] platelets,[13] and cellular damage,[14] as well as metabolic derangements.[15] With such a plethora of different effects attributable to FFP resuscitation, it is highly likely that this resuscitation strategy modulates cellular regulation relatively upstream, such as gene transcription.

Whether the effect of FFP resuscitation can be detected at the level of the gene transcriptome is unknown and constitutes the focus of this study. Using our previously validated large animal model of combined TBI and HS, we hypothesized that FFP resuscitation would be associated with modulation of brain gene expression profiles compared with normal saline and colloids (hetastarch), and that these gene alterations could also be associated with the clinically relevant end points of brain lesion size and swelling.

## METHODS

This study adhered to the guidelines stipulated in the Animal Welfare Act, as well as federal statutes for animal experiments. All experiments were performed under the supervision of a veterinarian and were approved by the Institutional Animal Care and Use Committee. This study represents secondary use of biomaterial retrieved from a previously published study.[10]

### Animal model

This porcine model of combined TBI and HS has been described in detail previously.[10] Briefly, 15 female Yorkshire swine (42.50 kg; Tufts Veterinary School) were used for this study. Animals were anesthetized using isoflurane and cannulations of the left femoral and internal jugular veins, as well as right and left femoral arteries, were performed using a cutdown technique. A 20-mm bur hole was made on the right side of the skull to expose the dura. This was used for the creation of TBI using a computer-controlled cortical impact device as described previously.[10] An additional 2-mm bur hole located 10 mm lateral and 10 mm anterior to the bregma was used for insertion of a combined intracranial pressure (ICP) and brain oxygenation measurement device.

After the cortical impact, animals were subjected to a controlled 40% hemorrhage, followed by 2 hours of shock, targeting a mean arterial pressure (MAP) of 35 mmHg. Animals were then resuscitated (n = 5/group) with FFP, normal saline, or hetastarch. Volumes of FFP and hetastarch equaled that of the shed blood, and normal saline was 3 times the volume of shed blood. Fresh frozen plasma was procured from healthy porcine donors, as described previously.[10] After resuscitation, animals were observed under anesthesia for 6 hours before sacrifice.

### Tissue sampling and RNA preparation

Tissue sampling and RNA preparation have been described in detail previously.[16] Briefly, brains were harvested immediately on sacrifice and sliced into 5-mm sections in the coronal plane. Brain slices were incubated in 2% 2,3,5 triphenyltetrazolium chloride to stain viable tissue. Lesion size was then calculated by the use of the ImageJ software package (NIH). The degree of brain swelling was assessed by comparing the ipsilateral and contralateral hemisphere volumes, as described previously.[10] Thirty-milligram pieces of tissues were obtained from the penumbra of the injury inferior to the lesion and homogenized. RNA was then extracted from the samples (RNeasy Mini Kit; Qiagen) and prepared for microarray analysis with the GeneChip WT Plus Reagent Kit (Affymetrix Inc) according to manufacturer instructions. RNA was then reverse transcribed to synthesize complementary DNA, as described previously,[16] before hybridization onto an Affymetrix porcine Gene ST 1.1 microarray. For each gene, the expression values were calculated using a multiarray mean. This consisted of background correction, quantile normalization, and summarization of the expression values.[16,17]