Sex and genetic associations with cerebrospinal fluid dopamine and metabolite production after severe traumatic brain injury

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Object. Dopamine (DA) pathways have been implicated in cognitive deficits after traumatic brain injury (TBI). Both sex and the dopamine transporter (DAT) 3′ variable number of tandem repeat polymorphism have been associated with differences in DAT protein density, and DAT protein affects both presynaptic DA release, through reverse transport, and DA reuptake. Catecholamines and associated metabolites are subject to autooxidation, resulting in the formation of reactive oxygen species that may contribute to subsequent oxidative injury. The purpose of this study was to determine associations between factors that affect DAT expression and cerebrospinal fluid (CSF) DA and metabolite levels after severe TBI.

Methods. Sixty-three patients with severe TBI (Glasgow Coma Scale score ≤ 8) were evaluated. The patients’ genotypes were obtained using previously banked samples of CSF, and serial CSF samples (416 samples) were used to evaluate DA and metabolite levels. High-performance liquid chromatography was used to determine CSF levels of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) during the first 5 days after injury.

Mixed-effects multivariate regression modeling revealed that patients with the DAT 10/10 genotype had higher CSF DA levels than patients with either the DAT 9/9 or DAT 9/10 genotypes (p = 0.009). Females with the DAT 10/10 genotype had higher CSF DA levels than females with the DAT 9/9 or DAT 9/10 genotypes, and sex was associated with higher DOPAC levels (p = 0.004). Inotrope administration also contributed to higher DA levels (p = 0.002).

Conclusions. In addition to systemic administration of DA, inherent factors such as sex and DAT genotype affect post-TBI CSF DA and DA metabolite levels, a phenomenon that may modulate susceptibility to DA-mediated oxidative injury.

Key Words: traumatic brain injury • cerebrospinal fluid • dopamine • homovanillic acid • dihydroxyphenylacetic acid • sex-based difference • genetic polymorphism

The catecholamine DA is considered an important neurotransmitter for a broad range of cognitive functions, including attention and executive control. Catecholamines, including DA and its metabolites, are subject to autooxidation, resulting in the formation of reactive oxy-

Abbreviations used in this paper: CBF = cerebral blood flow; COMT = catechol-O-methyltransferase; CPP = cerebral perfusion pressure; CSF = cerebrospinal fluid; CT = computed tomography; DA = dopamine; DAT = DA transporter; DOPAC = 3,4-dihydroxyphenylacetic acid; EVD = extraventricular drain; GCS = Glasgow Coma Scale; HPLC = high-performance liquid chromatography; HVA = homovanillic acid; ICP = intracranial pressure; MAO = monoamine oxidase; PCR = polymerase chain reaction; SEM = standard error of the mean; TBI = traumatic brain injury; VNTR = variable number tandem repeat.

Gen species. Traumatic brain injury results in several acute secondary injury cascades that lead to oxidative injury and cell death. Although not well studied, the biochemical properties of DA may contribute to oxidative injury after TBI. In addition, chronic biochemical disturbances occur after TBI. For example, chronic disruption of a number of DA-related proteins, as well as DA neurotransmission in the striatum, have been reported in experimental TBI models. Moreover, human studies using single-photon emission CT indicate that DAT expression is reduced chronically after TBI.

Dopamine transporter is a crucial protein in the regulation of DA transmission, playing a central role in determining the duration of action of DA by rapidly taking up extracellular DA into presynaptic terminals after release. Studies in animals lacking expression of the DAT gene sug-
Differences in the number of uptake sites with the 10-repeat allele enhancing the all CSF samples were snap frozen at the time of collection. In addition, DAT expression is modulated through polymorphisms associated with the DAT gene. The VNTR genetic variant for the human DAT gene (DAT1) is a 40-bp sequence located on chromosome 5p15.3, with the two most common polymorphisms including nine and 10 repeats. The two most frequent alleles, the nine-repeat and 10-repeat alleles, appear to differentially alter the transcription of DAT1, with the 10-repeat allele enhancing the transcription and increasing the amount of available transporter. The DAT1 gene is associated with attention deficit hyperactivity disorder, a syndrome related to altered DA function and characterized by inattention, impulsivity, and dyscontrol. Increased DAT binding has also been shown with single-photon emission CT to occur in those with attention deficit hyperactivity disorder, compared with controls, and individuals with DAT 9/10 heterozygosity have approximately 22% lower DAT binding capacity than DAT 10/10 homozygotes.

Estrogen exerts a significant influence over the regulation of DA systems through genomic and nongenomic mechanisms. Specifically, DAT binding site density has been shown to be higher in females than males in both animal and human studies. In addition, recent studies suggest the existence of sex-based differences in regional DAT expression in response to controlled cortical impact injury, with smaller postinjury reductions in DAT expression noted in females, compared with males.

Given the potential role of DA in contributing to secondary injury after TBI, the influence of both DAT genotype and sex on DAT regulation, and the critical influence of DAT in modulating both DA release and clearance, the objective of this study was to determine how these factors affected DA and associated metabolite levels in the CSF of patients with severe TBI. Our primary hypothesis was that inherent factors that increase DAT protein expression, such as sex and DAT 10/10 genotype, may increase CSF DA levels and affect DA metabolism after TBI.

Clinical Material and Methods

Study Population

Analyses for this study met with the approval of our center’s institutional review board. We evaluated 63 patients (15 female and 48 male patients; a total of 416 CSF samples) with severe TBI admitted to our Level 1 trauma center between 1995 and 2002. The 27 patients admitted between 1995 and 1998 were a subset of patients enrolled in a randomized controlled multicenter clinical trial evaluating moderate hypothermia (cooling to 32.5–33.5°C for a 48-hour period commencing within 8 hours of injury) after severe TBI. These patients met the following inclusion criteria: 1) age between 16 and 70 years; 2) initial GCS score less than or equal to 8; 3) positive CT findings for TBI; 4) placement of an EVD for ICP monitoring; and 5) a signed informed consent form from the next of kin. The 36 patients admitted between 1998 and 2002 received hypothermia treatment as part of their standard care (cooling to 32.5–33.5°C for a 24-hour period commencing within 8 hours of injury) if they met the following criteria: 1) an initial GCS score of 5 to 8; 2) age less than 46 years; 3) CT studies positive for TBI; and 4) placement of an EVD for standard-of-care ICP monitoring. In total, 28 patients received hypothermia and 35 patients remained normothermic during their acute care. Of those receiving hypothermia, 12 received treatment for 48 hours, and 16 received treatment for 24 hours.

Critical Care Management of Severe TBI

A diagnosis of severe TBI requiring aggressive management was determined by a trained neurosurgical physician, and patients received treatment consistent with the Brain Trauma Foundation’s Guidelines for the Management of Severe Head Injury. This treatment included placement of an EVD catheter, central line, and arterial line. All patients with severe TBI requiring aggressive therapy were screened for participation in CSF collection, because EVD placement is a part of standard care. Permission to collect CSF was obtained with an additional protocol approved by our center’s institutional review board.

The patients were treated in an algorithmic fashion to maintain the CBF pressure within normal parameters (<20 mm Hg), and CPP was maintained at greater than 60 mm Hg. If CPP was low, then inotropes were utilized to maintain mean arterial pressure greater than 90 mm Hg. Patients were treated with hypothermia using previously published protocols.

Cerebrospinal Fluid Sample Collection and Processing

Timed, fresh CSF samples were collected from a sterile port of the EVD every 4 hours during the initial 24 hours after injury and every 6 hours over the following 4 days. The EVD was present for clinical need; therefore, if a patient improved, the EVD was removed. To prevent protein breakdown, all CSF samples were snap frozen at the time of collection at −20°C and later processed for HPLC within 48 hours of collection. Aliquots of each processed sample were then stored at −80°C in cryoprotectant tubes.

Dopamine Transporter Genotyping

Genotyping for the DAT (SLC6A3) 3′-untranslated region VNTR was completed using DNA extracted from previously banked CSF samples. The DNA was extracted from CSF using a DNA extraction kit (Qiagen Corporation). Polymerase chain reaction primers flanked the 40-bp repetitive element in the 3′-untranslated region of the DAT1 gene. Thirty cycles of denaturation at 95°C for 1 minute, annealing at 62°C for 30 seconds, and extension at 72°C for 1 minute were used to amplify the 280–600-bp product. Primers used for this amplification were 5′-TGT-GGTTGTAAGGGAACGGCTTAGG-3′ and 5′-CCTGTCGAGGTTACGGCTCAAGC-3′. After PCR cycling, PCR products underwent gel electrophoresis on a 1% agarose/2% high-resolution agarose gel (NuSieve, Cambrex).
Corps) stained with ethidium bromide for DNA band detection. Genotype assignment was based on allele sizes. Alleles with either nine or 10 VNTRs are the most common in the general population, and patients were grouped based on the following genotypes: DAT 9/9, DAT 9/10, and DAT 10/10.

High-Performance Liquid Chromatography Methods

Forty-microliter aliquots of human CSF samples were mixed with 1.8 μL of 9.4 N perchloric acid and centrifuged at 15,000 g for 10 minutes, and a 25-μL aliquot of the supernatant aliquot was sampled. Dopamine, DOPAC, and HVA concentrations were detected by HPLC using electrochemical detection methods (ESA Biosciences, Inc.). The HPLC system consisted of a pump and an autosampler with cooler and a coulometric array detector (with two four-channel analytical cells). Analytes were separated on a C18 column (MC-150 column [3 mm × 15 cm], ESA Biosciences, Inc.) maintained at 31°C with a mobile phase flow of 0.6 mL/minute. Eight serial coulometric electrodes, maintained at 31°C, with applied potentials from −120 to +300 mV in 60-mV increments were used for the measurement of DA and its metabolites. The mobile phase was purchased commercially (MD-TM, ESA Biosciences, Inc.) to minimize variability. To calculate DA, DOPAC, and HVA concentrations, standard samples were prepared using known concentrations of DA, DOPAC, and HVA (Sigma). Study sample concentrations were compared with injected standards and were determined using commercially available data acquisition software (CoulArray for 32-bit Windows, ESA Biosciences, Inc.). Standards were also used to confirm a linear detector response for DA-spiked solutions across the concentration range of the study samples. Data for each analyte are presented in nanomolar concentrations (nmol/L). Normal CSF values for DA, DOPAC, and HVA have been reported in the literature as 2.08, 89.03, and 318.6 nmol/L, respectively.

Variables and Measures

Daily average CSF DA, DOPAC, and HVA levels over 5 consecutive days were examined for this study, and concentrations are reported as nanomole/liter. For some patients’ samples, DA levels were undetectable by using our experimental methods. For these samples, a value of 0.072 nmol/L was assigned. This value is the average of the three lowest detectable DA concentrations in our sample set, and this value was defined as the detection limit for DA in our sample set. We also evaluated GCS scores, age, sex, DAT genotype, time from injury, and hypothermia treatment with respect to their associations with DA and DA metabolites after severe TBI. The GCS scores were recorded after resuscitation and without the influence of paralytics. Patients having at least one DAT 9 allele (genotypes DAT 9/9 and DAT 9/10) were compared with DAT 10/10 homozygotes. To assess factors associated with metabolic turnover after TBI, DOPAC/DA and HVA/DA ratios were determined for all patient samples at each time point.

To assess the effect of systemic administration of dopaminergic inotropes on CSF concentrations of DA and its metabolites during treatment in the intensive care unit, we classified each patient daily regarding receipt of any systemic DA. We then determined if there was a significant relationship between daily receipt of DA and daily CSF DA or metabolite levels in a time-dependent manner.

Statistical Analysis

Summary statistics, including mean and SEM, were computed for all continuous variables. Frequency distributions were determined for categorical variables. Mean differences with continuous demographic variables were calculated using the Student t-test. Group comparisons using categorical data were evaluated using either the chi-square test or the Fisher exact test. We performed logarithmic data transformations for DA, DOPAC, and HVA concentrations to normalize the distribution of the data. Because the CSF outcome variables were measured at multiple time points (Days 1–5) for each patient, repeated measurement analyses were performed using a mixed-effects model with a compound symmetry covariance structure to test overall time effect within a group for a single CSF marker. With this technique, univariate and multivariate models were created to evaluate factors affecting CSF DA, DOPAC, and HVA levels. All independent variables were considered when constructing each multivariate model, and all potential interaction effects between time, inotrope administration, injury severity, sex, hypothermia treatment, and genotype group on each of the biomarkers studied were explored for each model. In the univariate models, adjusted (log scale) means are presented for each group for all categorical variables. In each of the multivariate models, the beta coefficient represents the direction and the average change in DA, DOPAC, and HVA concentrations and in DOPAC/DA and HVA/DA ratios for each unit change in the independent variables studied, with adjustment for all the other covariates. Graphic representation of adjusted means (raw scale) for each comparison group was extracted from each of the multivariate analyses. Commercially available SAS statistical software (SAS Institute) was used for all analyses, and a p value of 0.05 or less was considered statistically significant for all analyses.

Results

Description of the Patient Sample

Table 1 shows several descriptive statistics for the patient sample. The patients had a mean age of 31.49 ± 1.80 years, the median GCS score was 6, and 15 patients were female. Approximately 24% of the US DAT gene pool includes the nine-repeat allele, and approximately 76% includes the 10-repeat allele. The remaining 6% of the gene pool includes DAT alleles with other numbers of repeat units. Among the patients in the study, 71% of the DAT allele pool consisted of the 10-repeat allele, and 29% consisted of the nine-repeat allele, suggesting that our patient sample was similar to the national population with respect to this gene distribution. There were no significant sex-based differences with respect to the demographic data presented; however, a higher proportion of female patients in the study carried the DAT 10-repeat allele (p = 0.05).

Mean ± SEM DA levels for the 5-day data collection period were 1.76 ± 0.53 nmol/L on Day 1, 5.27 ± 4.02 nmol/L on Day 2, 3.01 ± 1.03 nmol/L on Day 3, 8.38 ± 3.94 nmol/L on Day 4, and 4.02 ± 1.80 nmol/L on Day 5. Compared with normal levels reported in the literature, the raw mean values for DA were elevated above normal
levels for 4 of the 5 days. Mean ± SEM DOPAC levels for the 5-day data collection period were 67.00 ± 7.95 nmol/L on Day 1, 75.09 ± 7.36 nmol/L on Day 2, 72.86 ± 9.78 nmol/L on Day 3, 58.88 ± 7.26 nmol/L on Day 4, and 41.56 ± 5.03 nmol/L on Day 5. These levels for DOPAC are qualitatively similar to normal values reported in the literature. Mean HVA levels for the 5-day data collection period were 1192.5 ± 95 nmol/L on Day 1, 1373.5 ± 92.9 nmol/L on Day 2, 1607.4 ± 118.6 nmol/L on Day 3, 1481.3 ± 101.8 nmol/L on Day 4, and 1307.9 ± 88.5 nmol/L on Day 5. These concentrations appear to be higher at all time points than normal values reported in the literature. Concentration ranges for each of these analytes were variable, with some individual sample values much above reported control values (0.06–222.1 nmol/L for DA, 1.47–334.6 nmol/L for DOPAC, and 20.41–4534.4 nmol/L for HVA). Analysis suggests that DA pressor support was administered during the CSF sample collection period for approximately 23.2% of the samples. Sixteen percent of all CSF samples were collected within 1 hour after systemic DA administration. The percentage of patients receiving systemic dopamine administration varied across days (21.1% on Day 1, 30.9% on Day 2, 25.0% on Day 3, 25.5% on Day 4, and 10.0% on Day 5).

**Univariate Analysis**

Table 2 presents the results of univariate analyses evaluating the relationship of each independent variable with analyte production over time. Patients with the DAT 10/10 genotype had higher CSF DA levels than those with either the DAT 9/9 or DAT 9/10 genotypes (p = 0.06). In addition, administration of dopaminergic inotropes was significantly associated with higher CSF DA levels (p = 0.003), and there was a trend for hypothermia to increase CSF DOPAC levels (p = 0.08). Levels of CSF HVA were significantly increased over time, compared with Day 1 values (p = 0.003). Inotrope administration was associated with a significant decrease in DOPAC/DA ratios (p = 0.01), and patients with the DAT 10/10 genotype had significantly lower HVA/DA ratios than patients with the DAT 9/9 or DAT 9/10 genotypes (p = 0.04).

**Multivariate Analysis**

Multivariate regression models describing factors that significantly affected CSF DA and its metabolites, with adjustment for all other independent variables, are presented in Table 3. The DA levels increased over time, and Day-1 levels were significantly lower than levels at Days 4 and 5 (p < 0.05 for all comparisons; Fig. 1 upper). The DAT genotype was a primary variable associated with CSF DA levels after TBI. Patients with the DAT 10/10 genotype had significantly higher DA levels than those with either the DAT 9/9 or the DAT 9/10 genotype (p = 0.0009). Administration of dopaminergic inotropes was associated with significantly higher CSF DA levels (p = 0.002). There was a trend for higher GCS scores to be significantly associated with lower CSF DA levels (p = 0.07). Age and treatment with hypothermia were not independently associated with CSF DA levels. The adjusted mean DA levels (raw scale) for selected comparison groups in this multivariate analysis are presented in Fig. 1 lower. There was a significant interaction between sex and DAT genotype with respect to CSF DA levels (p = 0.04). Female patients with the DAT 10/10 genotype had significantly higher DA levels than female patients with either the DAT 9/9 or DAT 9/10 genotype (p = 0.01), although no significant association between DAT genotype and DA level was found in the male patients. Also, among female patients with the DAT 10/10 genotype, there was a trend for higher DA levels, compared with male patients with this genotype (p = 0.097; Fig. 2).

Mean CSF DOPAC levels did not change significantly over the time period studied (Fig. 3 upper). Significantly lower DOPAC levels were found for each year increase in age (p = 0.05). In addition, the female patients had significantly higher DOPAC levels than the male patients (p = 0.004), and patients treated with hypothermia had higher DOPAC levels than those who were not treated with hypothermia (p = 0.05) (Table 3). Systemic DA administration and genotype group did not affect DOPAC levels. Adjusted mean DOPAC levels (raw scale) for each comparison group are presented in Fig. 3 lower. It is interesting to note that time after injury was significantly associated with HVA level. Figure 4 upper shows a continued increase in adjusted mean (raw scale) values over each day of the evaluation period. No other variables were significantly related to CSF HVA level. Adjusted mean values of HVA for each comparison group are presented in Fig. 4 lower. There was a significant decrease in adjusted means (raw
TABLE 2

Univariate repeated models used in the evaluation of the relationship between clinical characteristics in patients with severe TBI and DA analyte production after injury

<table>
<thead>
<tr>
<th>Variable</th>
<th>Log (DA)</th>
<th>Log (DOPAC)</th>
<th>Log (HVA)</th>
<th>Log (DOPAC/DA)</th>
<th>Log (HVA/DA)</th>
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<td>SEM</td>
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<td>p Value</td>
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* Outcome values represent the average of all analyte measures obtained within each day.
† Significantly different from mean Day 1 values (p < 0.05).
‡ Group 1: DAT 10/10 genotype; Group 2: DAT 9/10 and DAT 9/9 genotypes.
scale) for DOPAC/DA ratios over the study period (p = 0.05; Fig. 5 upper). In addition, DOPAC/DA ratios were significantly associated with GCS scores (p = 0.05). Higher metabolic ratios were associated with better GCS scores. In contrast, systemic administration of DA significantly decreased DOPAC/DA ratios (p = 0.0006). The adjusted mean values of DOPAC/DA for each comparison group are shown in Fig. 5 lower. There was no significant variation in HVA/DA ratios over time (Fig. 6 upper). Patients with better GCS scores had significantly higher ratios (p = 0.01). Furthermore, patients with the DAT 10/10 genotype (p = 0.02) and who received DA (p = 0.0009) had significantly lower HVA/DA ratios. The adjusted raw mean values of HVA/DA for each comparison group in the multivariate model are shown in Fig. 6 lower.

**Discussion**

Clinical CSF analysis studies have characterized several features of secondary injury cascades. In contrast, less emphasis has been placed on characterizing catecholamine levels after TBI. Previous work in experimental models has suggested transient regional increases in tissue DA concentrations after experimental TBI and return to baseline concentrations over time.35,37 In addition, the role of systemic DA concentration on physiological parameters has been evaluated after TBI.2,31,45 Early work evaluated CSF HVA accumulation in TBI patients, with variable results.44,51,57 However, the data presented here provide a comprehensive evaluation of factors affecting DA and metabolite accumulation in CSF acutely in a clinical population with TBI.

In the present study, we found that after severe TBI, patients’ CSF DA and HVA levels—but not DOPAC levels—were higher than reported control values. The largest elevations above the control values were observed for HVA and were roughly comparable to the DA and metabolite profile noted in a previous study in patients with subarachnoid hemorrhage, where, in CSF samples collected within...
48 hours of injury, mean values were 1.15 ± 0.7 nmol/L for DA, 37.36 ± 5.43 nmol/L for DOPAC, and 1810 ± 240 nmol/L for HVA. Porta and colleagues also have reported elevated HVA levels from ventricular CSF early after severe TBI. Homovanillic acid is a principal metabolite of DA after its conversion by COMT to 3-methyltyrosine. Alternatively, DOPAC is a major product of DA that is produced directly through MAO metabolism. Perhaps TBI differentially affects COMT activity, compared with MAO activity, in a manner that results in elevated HVA levels but not elevated DOPAC levels. Elevations in CSF HVA levels appear to be primarily the result of sustaining a TBI, as elevations over time are not affected by injury severity and do not vary relative to other factors that were analyzed. In contrast, innate factors such as sex and DAT genotype appear to affect relative CSF DA levels after TBI, and the TBI and systemic administration of DA are both likely contributors to levels that are higher than the reported control values.

The results of this study indicate that patients with the DAT 10/10 genotype had relatively higher CSF DA levels than those with either the DAT 9/9 or DAT 9/10 genotype but that genotype did not affect DA metabolite production. Previous studies have shown relationships between other monoaminergic genotypes and CSF monoamine/metabolite concentrations. Moreover, previous findings have suggested that individuals with the DAT 10/10 genotype have higher DAT binding densities than those with other genotypes. Dopamine release is known to occur through voltage-dependent and voltage-independent mechanisms, as well as reverse transport. In the setting of elevated intracellular Na⁺, carrier-mediated release (reverse transport) of monoamines, as well as increases in Ca²⁺ mediated vesicular release of monoamines, has been reported. In general, the contribution of TBI to elevations in CSF DA levels after TBI is likely multifactorial and is probably the result of increased Na⁺-mediated Ca²⁺-dependent exocytosis, increased reverse transport occurring from intracellular Na⁺ accumulation and/or excessive glutamate stimulation, and impaired reuptake. Our results suggest that relative increases in CSF DA accumulation for patients with the DAT 10/10 genotype, compared with those with the DAT 9/9 or DAT 9/10 genotype, were a result of an increased contribution of reverse transport-mediated DA release to overall DA accumulation.

Estrogen modulates actions within multiple neurotransmitter systems, including DA. The general consensus is that estrogen has an augmentative effect on DA neurotransmission and may be neuroprotective in models of neurodegenerative disease. Dopamine transporter densities are increased in females compared with males, and previous studies have found that hormone replacement therapy has a restorative effect on DAT density for menopausal women. In this study, CSF DA levels were significantly different for female patients in each DAT genotype group. Given the influence of estrogen on DAT density and dopaminergic A. K. Wagner et al.
transmission, it is possible that estrogen influenced the sex-related DAT genotype interaction noted in the CSF DA levels.

Although CSF DOPAC levels did not appear to be significantly increased above reported control values, female sex was associated with relatively higher DOPAC levels. Estrogen is known to downregulate COMT expression and activity, and estrogen’s effects are, in part, mediated through a COMT gene promoter sequence where gene transcription is limited in the presence of 17-β-estradiol. The blockade of COMT results in a shift from DA metabolism, through O-methylation, to oxidation, through the MAO pathway. Primarily extraneuronal in location, MAO-B constitutes the majority of the total MAO activity in the brain, and estrogen increases MAO-B activity. Given the influences of estrogen on COMT and MAO, higher DOPAC levels for female patients after TBI may be a result of the relative activities of each enzyme as a function of both hormones and injury. The lack of a sex-based effect on HVA levels after severe TBI may have occurred because of overall larger increases in HVA noted in the patients in our study and because MAO and COMT ultimately both contribute to HVA production. It is interesting to note that HVA levels continued to rise over time, indicating ongoing DA hypermetabolism.

Even though systemic administration of DA was reported for a minority of patients and samples collected, it was significantly related to higher DA levels and lower metabolic ratios. However, despite a lower frequency of DA administration by Day 5, CSF DA levels continued to rise over time, with Day 4 and Day 5 levels being significantly higher than Day 1 levels in the multivariate analysis. These results suggest that the effects of the TBI are a significant contributor to CSF DA levels over time. Systemic administration of DA helped maintain CPP in this cohort. In previous experimental work, systemic administration of DA has resulted in inconsistent increases in CBF in patients with TBI. However, in other studies, brain edema has increased in regions both ipsi- and contralateral to the injury site, and elevations in ICP have been reported with systemic DA. Moreover, the role of increased CSF DA levels, particularly with systemic DA administration, in oxidative injury and neurotoxicity after TBI remains unknown. However, conditions in which there is an increase in availability of DA and a decrease in antioxidant reserve may predispose patients to more oxidative injury. As such, the possibility remains that systemic DA administration may contribute to some aspects of secondary injury, despite its beneficial effect on maintenance of CPP. The trend for worse GCS scores to be related to higher DA levels and lower metabolic turnover in the multivariate analysis suggests that injury severity does play a role in the accumulation of CSF DA and is not simply a function of the inotropic support that is administered to more severely injured patients.

Both DA and DA metabolites have been implicated as neurotoxic agents in Parkinson disease. After TBI, accumulation of DA and its metabolites, particularly in DA-rich regions such as the striatum, may play a role in terminal degeneration, receptor alterations, and impaired neurotransmission. Furthermore, damage imparted to dopaminergic structures by DA and its metabolites may underlie the benefits of chronic DA agonist administration after TBI.

With exogenous DA administration increasing the CSF DA levels in these TBI patients and the potential for DA-mediated oxidative injury, the risk/benefit ratio of this compound for pressure support and CPP maintenance clinically...
may need to be explored in more detail. Hypothermia and age had significant effects on CSF DOPAC accumulation after severe TBI, with hypothermia increasing DOPAC accumulation and age being associated with decreased levels. Given the role of DOPAC in free radical formation, these findings may warrant future study.

Some study limitations should be considered when interpreting the findings of this study. One consideration is that we did not have control CSF samples to measure DA and metabolite levels with our own HPLC system, and literature-based reference values were used. In addition, the role of estrogen in mediating sex-based effects on DA and its metabolites was not directly measured. Future work prospectively evaluating the role of ovarian hormones on DA and metabolite production may be useful. Although we measured systemic inotrope administration during the CSF collection period, we did not specifically study the relationship of CSF biomarkers to other end points such as ICP, CPP, and CBF. Finally, assessing the relationship of CSF DA and DA metabolite levels with oxidative injury is a critical next step in this line of research.

Conclusions

Our data indicate that DA and DA metabolites are elevated in a clinical population with severe TBI. Endogenous as well as exogenous factors primarily associated with these elevations, such as DAT genotype, sex, and the systemic administration of DA, may influence DA-mediated damage after TBI. Future work should focus on the contribution of DA levels to oxidative injury post-TBI and the role of DA as a biological marker for recovery. In addition, research on the effect of hormones and other dopaminergic genotypes on CSF DA and DA metabolite levels is warranted.

References


A. K. Wagner et al.
Sex, genetics, and dopamine production after severe TBI


