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Exposure of Cyclosporin A in Whole Blood, Cerebral Spinal Fluid, and Brain Extracellular Fluid Dialysate in Adults with Traumatic Brain Injury

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Abstract

Cyclosporin A (CsA), an immunosuppressive medication traditionally used in the prevention of post-transplant rejection, is a promising neuroprotective agent for traumatic brain injury (TBI). Preliminary studies in animals and humans describe the efficacy and safety of CsA when administered following neurotrauma. The objective of this study is to describe CsA exposure in adults with severe TBI by assessing concentrations in whole blood, cerebrospinal fluid (CSF), and brain extracellular fluid (ECF) dialysate as measured by brain microdialysis. Severe TBI patients were enrolled in a randomized controlled trial following the written informed consent of their legal guardians. Patients received either CsA 5 mg/kg as a continuous infusion over 24 h, or matching placebo. Noncompartmental exposure analyses were performed using CsA concentrations in whole blood, CSF, and ECF dialysate. There were 37 patients randomized to the CsA arm of the trial and included in this exposure analysis. CsA was detected in the ECF dialysate and CSF at a fraction of the whole blood concentration. Mean CsA maximum concentrations were achieved at 24 and 30 h from the start of the 24 h infusion, in the CSF and ECF dialysate, respectively. A correlation was found between ECF dialysate and CSF concentrations. CsA was detected in the blood, CSF, and brain ECF dialysate. CsA exposure characteristic differences exist for whole blood, CSF, and ECF dialysate in severe TBI patients when administered as a continuous intravenous infusion. These exposure characteristics should be used for safer CsA dose optimization to achieve target CsA concentrations for neuroprotection in future TBI studies.

Key words: cyclosporin; neuroprotection; TBI

Introduction

Traumatic brain injury (TBI) is the leading cause of death and disability for children and adults < 44 years of age. At least 5,300,000 people in the United States are currently living with disabilities resulting from a TBI.[1] Given the serious ramifications of neurotrauma, and the inability to reverse the primary axonal injury, research is focused on the development of pharmacological modalities that attenuate the devastating secondary sequelae of trauma. To date, no pharmacological agents have been shown to definitively improve outcomes after TBI. The most promising treatment strategies are neuroprotective agents that reduce ischemia, inflammation, free radical damage, and metabolic derangements in brain tissue.[2]

Cyclosporin A (CsA), an agent traditionally used as an immunosuppressive medication in the prevention of post-transplant rejection, has several mechanisms of action that make it a promising neuroprotective agent for TBI. First, the secondary damage following trauma may be related to excessive activation of *N*-methyl-D-aspartate (NMDA) receptors caused by elevated glutamate concentrations in the brain.[3] NMDA stimulation leads to an intracellular influx of calcium, which initiates the cascade of reactive oxygen species generation and adenosine triphosphate (ATP) metabolic derangements. Second, mitochondria sequester calcium during cellular hypercalcemia to maintain calcium homeostasis, and are thus intimately involved in the pathophysiology of neuronal death following TBI.[3,4] When mitochondria approach their ''high

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conductance state'' because of large changes in pH and the presence of large amounts of intracellular calcium, then the mitochondrial permeability transition pore opens in an irreversible manner, allowing calcium to flood the mitochondrion and causing mitochondrial swelling, efflux of free radicals, and thus, failure of mitochondrial function.[5–7] This occurs in contrast to the ''low conductance opening'' that allows the mitochondrion to continuously ''cycle'' small amounts of calcium in and out of the inner mitochondrial membrane in order to carry out oxidative metabolism.[6] CsA acts to prevent mitochondrial dysfunction in the setting of elevated cytosolic calcium concentrations by inhibiting opening of the mitochondrial permeability transition pore.[8] The end-point of inhibition is maintenance of the mitochondrial membrane potential and prevention of calcium efflux out of the mitochondria. In the absence of CsA, activation of the mitochondrial permeability transition pore is associated with uncoupling of oxidative phosphorylation, osmotic swelling, and lysing of the mitochondrial membrane.[3,8–12]

CsA has demonstrated efficacy and safety in *in vivo* animal and preliminary human studies of TBI[2,4,7,11,13] However, no studies to date have reported the exposure of CsA in multiple biofluids following neurotrauma. The aim of the current study is to describe the pharmacokinetic (PK) parameters of CsA in adults with TBI by sampling drug concentrations in the whole blood, cerebrospinal fluid (CSF), and brain extracellular fluid (ECF) dialysate. This will allow safer dose optimization for neuroprotection, given that a rapid neuronal exposure to CsA after TBI is the goal, as shown in animal studies.[3,14–18]

Methods

The study was a prospective, placebo-controlled, dual-center, randomized controlled trial evaluating the exposure and safety of CsA in severe TBI patients. The study was approved by the Institutional Review Board at Virginia Commonwealth University and the University of Florida. Consent was obtained from the legally authorized representative or next of kin for each subject. Patients enrolled were > 16 years of age with a severe TBI, defined as a Glasgow Coma Score (GCS) of 3–8. All patients received a ventriculostomy and a microdialysis catheter unless contraindicated because of underlying coagulopathy. Patients were excluded if they had bilateral fixed and dilated pupils or if they demonstrated evidence of renal dysfunction (blood urea nitrogen [BUN] > 20 mg/dL, creatinine > 1.3 mg/dL), hepatic dysfunction, history of malignancy, pregnancy, immunosuppression, known life-threatening disease prior to trauma, or current or prior use of another investigational agent within 30 days of enrollment. Patients with elevated intracranial pressures were managed with both continuous and intermittent drainage from the ventriculostomy catheter as deemed clinically necessary. Patients were randomized in a 3:1 ratio to receive either CsA 5 mg/kg diluted in 250 mL of D5W as a 24 h continuous infusion, or matching placebo. All doses were administered within 12 h of the primary injury. Impact acceleration murine studies have demonstrated a narrow therapeutic range of CsA after TBI. A dose of 10 mg/kg IV dose was deemed the most effective in terms of decreasing the mean density of damaged axons; however, a dose of 50 mg/kg was both toxic and ineffective at reducing axonal injury.[19] A CsA dose of 5 mg/kg was chosen, based upon safe plasma levels, abstracted from the transplant literature, and also based upon neuroprotective effects in animal studies. Our study protocol consisted of brief administration (24 h) of a therapeutic dose of CsA within the recognized dosing range of 5–6 mg/kg. Furthermore, 5 mg/kg was the maximal ''safe dose'' used in other human TBI studies,[2] and a dose of 2.5 mg/kg was used by others for attenuating myocardial reperfusion injury after myocardial infarction in humans.[20] We were especially concerned with avoiding a dose that could induce dangerous acute immune suppression in this study. The safety and tolerability of this CsA dosing regimen have been reported previously, as part of the same trial.[11]

CsA concentrations were determined in whole blood, CSF, and ECF, obtained via microdialysis. Whole blood, CSF, and ECF dialysate samples were drawn at 1 h before CsA administration, and thereafter at 75 min, 4 h, 6 h, 8 h, 12 h, 24 h, 36 h, 48 h, and 72 h after administration.

Assay methods

Whole blood CsA concentrations were measured from blood samples (0.5 mL) using a commercial fluorescence polarization immunoassay (TDx immunoassay) by the hospital clinical transplant laboratory. The CsA lower level of quantitation (LLOQ) of the FPIA assay is 25 μg/L, and the upper level is 1500 μg/L. CSF samples were drawn from the ventriculostomy catheter to a total volume of 0.5 mL per sample. Brain microdialysis was performed via a dialysis probe with a 10 mm long flexible membrane and a molecular weight cutoff of 20,000 Da (CMA 70,CMA/Microdialysis, Sweden), surgically inserted into the right frontal brain parenchyma through a double lumen skull bolt. The probes were perfused at 2 μL/min, using sterile 0.9% saline, through a precision microdialysis pump (CMA 107, CMA/Microdialysis, Sweden). CMA 70 microdialysis probe is made out of biocompatible polyurethane tubing (OD 1.0 mm), a poplyurethane shaft (OD 0.9 mm) and a polyamide membrane (OD 0.6 mm).[21,22] The 20 kDa cutoff membrane microdialysis probes were the only commercially available United States Food and Drug Administration (FDA) approved membranes for use in the human brain at the time the study was performed. Recently, a new catheter, CMA 71, with a molecular cutoff of 100 kDa, has become available and is currently undergoing investigation in Europe, for the measurement of cytokines, neurotrophic factors, and biomarkers.[23,24] To reduce and control the protein adsorption while retaining the performance of the microdialysis catheter, new methods (tri-block copolymer Poloxamer 407 surface-modified microdialysis) are currently being investigated *in vitro*, and show great potential for studying complex protein adsorption systems.[25] Samples (150 μL volume) were analyzed with the CMA 600 Microdialysis Analyzer (CMA/Microdialysis, Sweden). CSF and ECF dialysate were analyzed with high performance liquid chromatography-mass spectrometry (HPLC-MS).[26] The CsA LLOQ of the HPLC-MS is 0.5 μg/L for the CSF and 0.025 μg/L for the ECF dialysate. The methods for these assays and microdialysate recovery have been previously published.[26]

Exposure analysis

Non-compartmental PK analyses were conducted to determine the area under the concentration-time curve (AUC_∞), maximally attained concentration, or peak (C_{max}), time to peak (T_{max}), total clearance (Cl_{tot}), half-life ($t_{1/2}$), and volume of distribution at steady state (Vd_{ss}). The following equations were used to calculate exposure characteristics:

$$\text{AUCtrap(tn)} = \text{sum i} = 1 \text{ to n}[(\text{ci} + \text{ci} - 1)^*(\text{ti} - \text{ti} - 1)/2]$$

$$\text{CL} = \text{Dose}/\text{AUC}\infty$$

$$\text{t}^{1/2} \text{ term} = \ln(2)/\lambda$$

$$\text{Vdss} = \text{CL}^*\text{MRTsys}$$

In calculating parameters for CSF and ECF dialysate, values that were below the LLOQ were excluded from the analysis if there were measurable concentrations before and after the time point, or were given a value of zero if they were at the end of the data set. To characterize the terminal slope of the CsA concentrations, a value of one half the LLOQ was assigned to the concentrations that were below the LLOQ.

Table 1. Patient Demographics

Patient demographics	*(n = 37)*
Age, mean ± SD (years)	34 ± 16
Gender, % male	81
Dose, mean mg/24 h ± SD	368 ± 60
GCS on admit, median	4
GCS 3–4	23 (62%)
GCS 5–6	8 (22%)
GCS 7–8	6 (16%)
Type of Injury, *n* (%)	
Focal	13 (36)
Diffuse	23 (64)

GCS, Glasgow Coma Score.

Statistical analysis

Descriptive statistics were used to describe the exposure characteristics of CsA. Data are presented as mean and standard deviation or median and interquartile range.

Results

Fifty patients were enrolled in the trial from January 2003 through November 2004, of which 37 received CsA and 13 received placebo. Only patients who received CsA are included in this exposure analysis. The majority of patients were young males with a diffuse axonal injury (Table 1). There was one whole blood and one CSF patient sample that yielded unreliable results, and these samples were not included in the respective kinetic analyses. The exposure characteristics of CsA in whole blood, CSF, and ECF in severe TBI patients are summarized in Tables 2, 3, and 4, respectively. Average CsA concentrations in the blood, CSF, and ECF dialysate over time are depicted in Figure 1. CSF exposure achieved 0.37% of whole blood AUC, whereas ECF dialysate exposure achieved 0.04% of whole blood AUC. Two patients achieved a peak whole blood CsA concentration that exceeded the upper limit of quantitation; these concentrations were reported as 1500 μg/L. The ECF dialysate concentration as a function of CSF concentration is presented in Figure 2, which demonstrated correlation between the two biofluids ($r^2 = 0.651$).

One whole brain tissue sample was available for analysis during the study period; CsA concentration was determined by HPLC-MS. CsA was detected in brain tissue in this patient at a concentration of 46.9 ng/g.

Discussion

CsA has demonstrated efficacy in attenuating cortical injury and improving neurological outcomes following neurotrauma. It has been shown to decrease ischemia-reperfusion injury in animal models, as assessed by cortical lesion size.[4,7] A prospective study utilizing a fluid percussion injury model in rats demonstrated that a 3 h infusion of CsA administered prior to TBI reduced motor and cognitive deficits after induced TBI.[13] A randomized, placebo-controlled dose escalation trial of CsA administered within 8 h of TBI in adults demonstrated a dose-dependent improvement in outcomes as assessed by the Glascow Outcomes Scale at 3 and 6 months post-injury.[2] Published literature to date does not reveal a significant increase in adverse events or laboratory abnormalities associated with the use of CsA compared with placebo, despite its historically narrow therapeutic index.[2,11] The previously published safety analysis from this trial demonstrated that patients treated with CsA had a mean creatinine and BUN within normal limits, and no statistically significant difference was noted in liver function tests, hemoglobin, or platelets compared to placebo.[11] The safety profile of CsA may be improved by PK studies that allow for precise determination of dosing strategies to achieve optimal therapeutic serum concentrations for neuroprotection in TBI patients.

Extrapolation of PK parameters from transplant patients or healthy volunteers may not be accurate for TBI patients because of metabolic and physiologic alterations following injury.[27] Previous studies have demonstrated that whole blood clearance, volume of distribution, and terminal half-life values are larger than in normal patients.[9] Animal models have demonstrated a steady increase in CYP3A activity following brain injury, with activity increasing up to 91% at 2 weeks in a rat model of blunt trauma.[28] In addition, disruption of the blood–brain barrier (BBB) following TBI may allow for increased concentrations of CsA in CSF as compared with normal controls.[13,29] In healthy adults, high protein binding and p-glycoprotein efflux pumps limit the BBB penetration of CsA. This study validated that the CsA volume of distribution (Vd) in whole blood was higher than reported values in healthy volunteers (Vd: 1.2 L/kg), and lower than in studies of renal transplant recipients Vd: 4.5 L/kg. Our results indicate a smaller volume of distribution and a lower clearance based on CsA whole blood concentrations as compared with the results of a prospective, randomized, placebo-controlled intermittent dose escalation study

Table 2. Exposure of Cyclosporin A in Whole Blood ($N = 36$)

	*AUC (mcg*h/L)*	*Half-life (h)*	*C_{max} (μg/L)*	*T_{max} (h)*	*CL (mL/min)*	*V_d ss (L/kg)*
Mean	18435	4.7	697	14	351	2.00
Standard deviation	5465	2.6	262	8	93	1.21

AUC, area under the curve; C_{max}, maximum concentration; T_{max}, time to maximum concentration; CL, clearance; Vd ss, volume of distribution at steady state.

Table 3. Exposure of Cyclosporin A in Cerebrospinal Fluid ($N = 28$)

	*AUC (mcg*h/L)*	*Half-life (h)*	*C_{max} (μg/L)*	*T_{max} (h)*
Mean	68	5.6	2.23	24
Standard deviation	76	6.9	2.37	13

AUC,area under the curve; C_{max}, maximum concentration; T_{max}, time to maximum concentration.

Table 4. Exposure of Cyclosporin A in Brain Extracellular Fluid Dialysate ($N = 21$)

	*AUC (mcg*h/L)*	*Half-life (h)*	*C_{max} (μg/L)*	*T_{max} (h)*
Mean	7.5	10.9	0.14	30
Standard deviation	9.4	21.7	0.14	29

AUC, area under the curve; C_{max}, maximum concentration; T_{max}, time to maximum concentration.

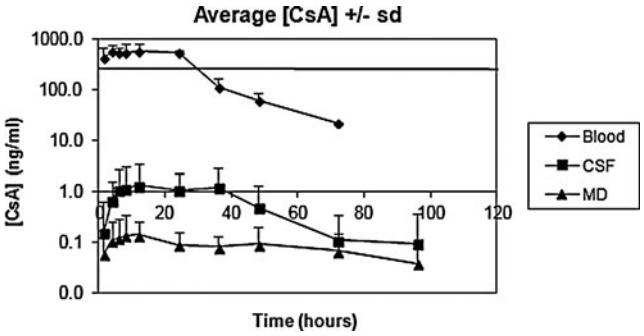

FIG. 1. Cyclosporin A concentrations in the blood, cerebrospinal fluid, and brain extracellular fluid dialysate in brain injured patients. Average concentrations are log transformed and error bars represent the standard deviation.

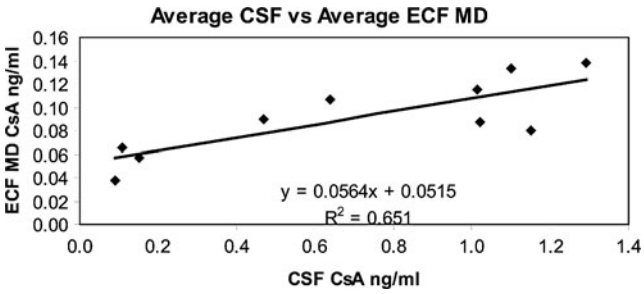

FIG. 2. Average brain extracellular fluid dialysate concentration as a function of average cerebrospinal fluid concentration at each time point.

conducted in severe TBI patients.[9] However, the AUC and C_{max} in our study were comparable to the 1.25 mg/kg dose in this study. The PK results of the dose escalation study are summarized in Table 5.[9] The reason for this discrepancy is unknown. The PK of CsA may be altered by several factors, including concomitant medications and disease states. Several medications that may be employed in TBI patients have PK interactions with CsA, including the hepatic enzyme inducers phenobarbital and phenytoin. In addition, CsA is extensively metabolized by cytochrome P450 (CYP)3A, which is subject to variability given the presence of genetic polymorphisms in CYP3A4 and CYP3A5.[34,35] Differences in concomitant medications or genetic polymorphisms may explain the variability in CsA exposure and PK parameters. In addition, different techniques in sampling and PK analysis may have created variability in results.

The present study may also have been influenced by variable body weights of study subjects. Although CsA is theoretically expected to have an increased volume of distribution in obesity because of its lipophilicity, studies of renal transplant patients have not demonstrated an increased volume of distribution at steady state in obese patients after normalization with ideal body weight.[36] Many authors have suggested that CsA be dosed on the basis of ideal body weight rather than actual body weight.[36] Doses in our study ranged from 225 mg daily to 475 mg daily on the basis of actual body weight, resulting in a dosing range from 4.5 to 7.3 mg/kg based on patients' ideal body weight. Differences in weight-based dosing may have led to increased concentrations in obese patients compared with those whose actual body weight approximated their ideal body weight.

CsA pententration into the CSF was minimal when compared with whole blood concentrations, although there is no clear CsA therapeutic range for neuroprotection after trauma. Previous studies have indicated a biphasic opening of the BBB in the ipsilateral hippocampus following neurotrauma, peaking within 3 h of injury and again at 24–48 h post-injury.[29] These data indicate that there may be a variable time course of BBB penetration that may influence CsA CSF and ECF concentrations; this remains to be fully elucidated in humans. In our study, CsA peak concentrations in whole blood occurred at a mean of 14 h after the start of the infusion, whereas peak concentrations in the CSF and ECF dialysate did not occur until a mean of 24–30 h after the start of the infusion, respectively. Our study's administration of CsA within 12 h post-injury for a total of 24 h may not have been during a period of maximal BBB disruption for all patients. These exposure differences found in this study can be used to help optimize dosing strategies to achieve target concentrations at the site of action.

PK analysis determining whether and to what extent the compound penetrated into CSF, and brain interstitial fluid as measured in the dialysate, is a novel area for evaluation of brain penetration, and could help in evaluating a drug's effect. Demonstration of the presence of drug in the ECF dialysate is important, especially for drugs whose potential neuroprotective role is being tested. Measurement of drug concentration in the ECF dialysate can provide a valuable feedback method to infer the concentration of the drug in the brain and thus to understand the *in vivo* dosage necessary to provide the desired neurochemical effect. In our study, differences were found in CsA CSF and ECF dialysate concentrations, as the blood-choroid plexus dynamics are not the same as the BBB. It is also possible that insertion of the microdialysis probe locally disrupts the BBB and can influence ECF concentrations over a short period of time.[37,38] Although it is unknown what the exact CsA concentrations are in the ECF; when evaluating the concentrations in the dialysate, there was a linear correlation with the CsA CSF

Table 5. Previously Reported Cyclosporin A Pharmacokinetics in Whole Blood in Traumatic Brain Injury Patients

	*AUC (mcg*h/L) Mean*	*β half-life (h)*	*Predicted C_{max} (μg/L)*	*C_{min} at 72 h (μg/L)*	*CL (mL/min/kg)*	*V_d ss (L/kg)*
Empey 2006 $n=24$	Whole blood:		Whole blood:			
0.625 mg/kg/dose q12h	9840	13.9	398		6.55	4.02
1.25 mg/kg/dose q12h	18300	19.3	645		7.18	6.10
2.5 mg/kg/dose q12h	32500	18.6	1300		7.5	7.62
Hatton 2008 $n=40$						
0.625 mg/kg/dose q12h				82		
1.25 mg/kg/dose q12h				116		
2.5 mg/kg/dose q12h				193		

AUC, area under the curve; $C_{max,}$ maximum concentration; C_{min}, minimum concentration; CL; clearance; Vd ss; volume of distribution at steady state.

concentrations. Therefore, it may be feasible to estimate the amount of CsA in the ECF based on CSF concentrations, which are more readily available in patients with a ventriculostomy catheter. This correlation should be validated in future studies using advanced microdialysis techniques that can confirm ECF concentrations. Animal studies have indicated that a brain parenchymal concentration of 0.898 μM is the most effective at attenuating neuronal injury.[19] This concentration converts to 1079 μg/L which is approximately double the mean whole blood peak concentrations found in this study. As the ECF microdialysate and CSF concentrations are only a fraction of the whole blood concentration, it is questionable whether adequate CsA doses were chosen or if there were other mechanisms by which CsA was exerting a neuroprotective effect, such as on the cerebrovasculature. An effective CsA concentration in humans has yet to be elucidated. Further analyses are being conducted to determine CsA dose-response relationships in TBI patients.

The exposure characteristics of CsA in TBI patients in this study were as expected based on its biochemical properties. The total blood clearance reflects that of a low extraction ratio drug, as previously reported in the literature. The large volume of distribution observed is suggestive of a significant degree of tissue binding and transporter involvement of CsA. As discussed, there was a large inter-patient variability in this study and exposure differences compared with previously reported literature, which may be related to differences in sampling technique or small sample size.

Limitations

There are several limitations to this evaluation. Although the administration of a 24 h continuous infusion of CsA limited the potential for side effects, it may have prevented some subjects from reaching steady state concentrations of CsA in all biological fluids. The concentration of CsA in the brain is continuously changing because of intracellular shifts, BBB permeability changes over time, and p-glycoprotein efflux pumps. We also recognize that, as with any clinical study collecting CSF samples, there is a potential limitation related to drainage method (continuous or intermittent), which has been discussed in the literature, and theoretically, may alter protein recovery.[38] Nevertheless, this limitation is common to all clinical studies collecting CSF samples, as continuous or intermittent CSF drainage may be required for clinical reasons (in response to increases in intracranial pressure) and cannot be prespecified for studies. Given the extensive binding of CsA to microdialysis tubing and filters, it is expected that only a small amount of CsA will be recovered by microdialysis probes in each patient; the protocol did not allow for determination of recovery by the probe in individual patients. In addition, several patients had peak whole blood values that reached the upper level of the assay at 1500 μg/L. It is unknown to what extent this limitation impacted calculation of true exposure characteristics.

Future directions

This study confirms that CsA, given via a 24 h continuous infusion, penetrates into the CSF, ECF as measured via brain microdialysis, and brain tissue. However, differences in exposure characteristics exist and cannot be ignored. Implications of these results on future study trial design are twofold. First, dosing strategies need to be determined based on the targeted site of action for neuroprotection, as differences in exposure characteristics exist between blood, CSF, and ECF (as measured by microdialysis). Second, until therapeutic concentrations are determined for CsA in TBI patients, dosing will most likely be limited by adverse effects. Additional studies are needed to determine associations between exposure characteristics and clinical outcomes so that CsA dosing can be safely optimized for neuroprotection in TBI patients.

Conclusion

This is the first published report of CsA exposure characteristics in the blood, CSF, and brain ECF dialysate in severe TBI patients. CsA concentrations were detected in all three biofluids analyzed. A correlation of CsA concentrations was found between CSF and ECF dialysate, which may provide a feasible way to determine if ECF concentrations of CsA are adequate to provide neuroprotection without the need for microdialysis. Future CsA neuroprotection studies should be designed based on exposure characteristics to safely optimize dosing strategies.

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Author Disclosure Statement

No competing financial interests exist.

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