

Original Article

Curcumin attenuates sensorimotor deficits induced by selective neuronal loss: A study in middle cerebral artery occlusion rat model of transient ischemic stroke.

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Abstract

Background: Ischemic stroke is the most prevalent and major pathological stroke type. Increased systemic oxidative stress is a crucial underlying pathophysiological mechanism of ischemic stroke. In this regard, therapeutic interventions targeting oxidative stress management would be a promising avenue. Curcumin is well recognized to counteract various pathologies effectively. The present study was designed to investigate the neuroprotective effects of curcumin against ischemia-reperfusion injury-induced selective neuronal damage.

Methodology: Present study employed an ischemic brain injury model induced by middle cerebral artery occlusion (MCAO) in Wistar albino rats. Experimental groups (n=36; age-matched, female, weighing 200-240 g) were control, sham-operated, MCAO (15 min occlusion + 24 hrs. reperfusion) and curcumin-treated pre-stroke group (received curcumin 300 mg/kg body weight/day, I/P, for 30 days followed by MCAO). With the completion of the experimental protocol, sensorimotor deficits were examined by behavioral tests, including neurological deficit score, foot fault test, forelimb placing test, corner turn test, and wire hanging test.

Results: Compared to MCAO rats, curcumin-treated MCAO rats showed better neurological functioning ($p < 0.05$) as exhibited by substantial reductions in neurological score. Compared with stroke group, curcumin treated group showed better functional outcomes as displayed by improved scores in foot fault index, and forelimb placing test ($p < 0.01$), fewer left turns ($p < 0.01$) in corner turn test, and prolonged grip latency in wire hanging test ($p < 0.01$).

Conclusion: The present study showed that designed curcumin treatment effectively manages MCAO-induced sensorimotor dysfunction by preventing selective neuronal loss after the ischemic attack, attributed to its antioxidant, anti-apoptotic, and anti-inflammatory potentials.

Keywords

Curcumin, Sensorimotor Deficit, Middle Cerebral Artery Occlusion, Ischemic Stroke.



Introduction

Stroke, a neurological disorder, results from compromised cerebral perfusion either because of hemorrhage or ischemia^{1,2}. Approximately 85 % of all stroke cases are of ischemic origin². Globally, stroke is the second leading cause of death and the third leading cause of complex disabilities with cognitive impairments^{3,4}. Among ischemic stroke incidences, transient ischemic attack (TIA) accounts for 20 % of cases associated with high odds of behavioral and cognitive impairments and sensorimotor deficits⁵. Clinically, TIA is characterized by a transient episode of focal neurological signs which lasts less than 24 hrs and without tissue injury or pannecrosis (acute infarction)^{6,7}. TIAs essentially result in a selective neuronal loss (SNL), a patchy (single) neuronal loss with the preserved extracellular matrix and without vascular cell death^{6,8} usually injuring the hippocampus's neurons its associated areas, higher cortical regions, and striatum / sensorimotor cortex⁹. The underlying pathophysiology of ischemic stroke (IS) is based on reoxygenation (ischemia-reperfusion) injury⁹. Tissue ischemia initiates the complex sequelae of the cellular process including microglial activation, glutamate excitotoxicity, apoptosis, oxidative stress, and disintegrated blood-brain barrier. Subsequent reperfusion of ischemic tissue paradoxically initiates reactive oxygen species (ROS) production and neuroinflammation¹⁰⁻¹².

Hence stroke survivors specifically post-TIA, patients suffered a wide array of neurological deficits (headache, vertigo, nausea, blurred vision, convulsions, and altered consciousness), sensorimotor deficits (tremors, poor muscle coordination, and paresis or complete paralysis), and dysfunctioning of higher cortical regions (memory, speech, and other cognitive disturbances)⁵.

Therefore, post-stroke functional assessment in experimental animal models could provide a valuable understanding of neurological dysfunction's underlying pathophysiology and investigate the potential effects of therapeutic agents and rehabilitation efforts. In this regard, the

middle cerebral artery occlusion (MCAO) ischemic stroke rodent model is a popular reproducible model used to study the IS-associated neuronal damage, sensorimotor deficits as well as for preclinical stroke trials¹³.

Two treatment approaches are currently available for ischemic stroke patients, intravenous thrombolysis and endovascular (mechanical) recanalization^{1,3}. For the former treatment option, the only approved drug is tissue plasminogen activator (tPA), which has a minimal therapeutic window of 3 to 4.5 hrs¹⁴, and for the latter, very few patients qualify for mechanical thrombectomy^{1,3,15} due to these therapeutic tribulations, pharmacological interventions are limited so far.

Among nonpharmaceutical interventions, natural products have long been proven as potential therapeutic agents attributable to their antioxidant properties^{14,16}. Turmeric or *Curcuma longa* Linn is a well-known culinary spice and a medicinal herb used in the traditional medicine system for decades. Curcumin, a bioactive constituent, extracted usually from the turmeric plant roots reported to possess antioxidants, anti-apoptotic, anti-inflammatory, anti-diabetic, and hypolipidemic therapeutic potentials¹⁷⁻¹⁸ hence known to exert protective effects on various physiological systems including the nervous system^{16,19,20}. Therefore, this study aimed to investigate the neuroprotective effect of curcumin administration against SNL-induced sensorimotor deficits in the MCAO rat model of transient focal cerebral ischemia.

Methodology

Study Design

Experimental animal research study conducted at the Department of Physiology, University of Karachi.

Ethics Statement

The departmental research committee approved all experimental procedures, Department of Physiology, University of Karachi, in accordance to the National Institutes of Health's Guide for the care and use of laboratory animals, and are

reported according to the ARRIVE (Animal Research: Reporting of in Vivo Experiments) guidelines²¹.

Experimental Animals

Female Wistar albino rats (body weight: 220 ± 20 g, age: 5-6 weeks old), purchased from International Centre of Chemical and Biological Sciences, University of Karachi (Karachi, Pakistan). Animals were housed at Animal Care Facility, Department of Physiology, University of Karachi. Animals kept under 12 hrs light / 12 hrs dark cycle, temperature-controlled ($23 \pm 2^\circ\text{C}$), ventilated room and fed with standard laboratory diet and water ad libitum.

Experimental Groups

A total of 36 animals were used in this study. After one week of acclimatization, animals were randomly divided into four experimental groups as:

- Control group (n=9)
- Sham-operated group (n=9)
- MCAO group (n=9), stroke-induced group
- MCAO+CUR group (n=9), stroke-induced group pre-treated with curcumin

All animals received a standard laboratory diet and water ad libitum throughout the study protocol. Animals from all experimental groups were evaluated for sensorimotor changes at the end of the experimental period (24 hrs. after MCAO and sham surgery).

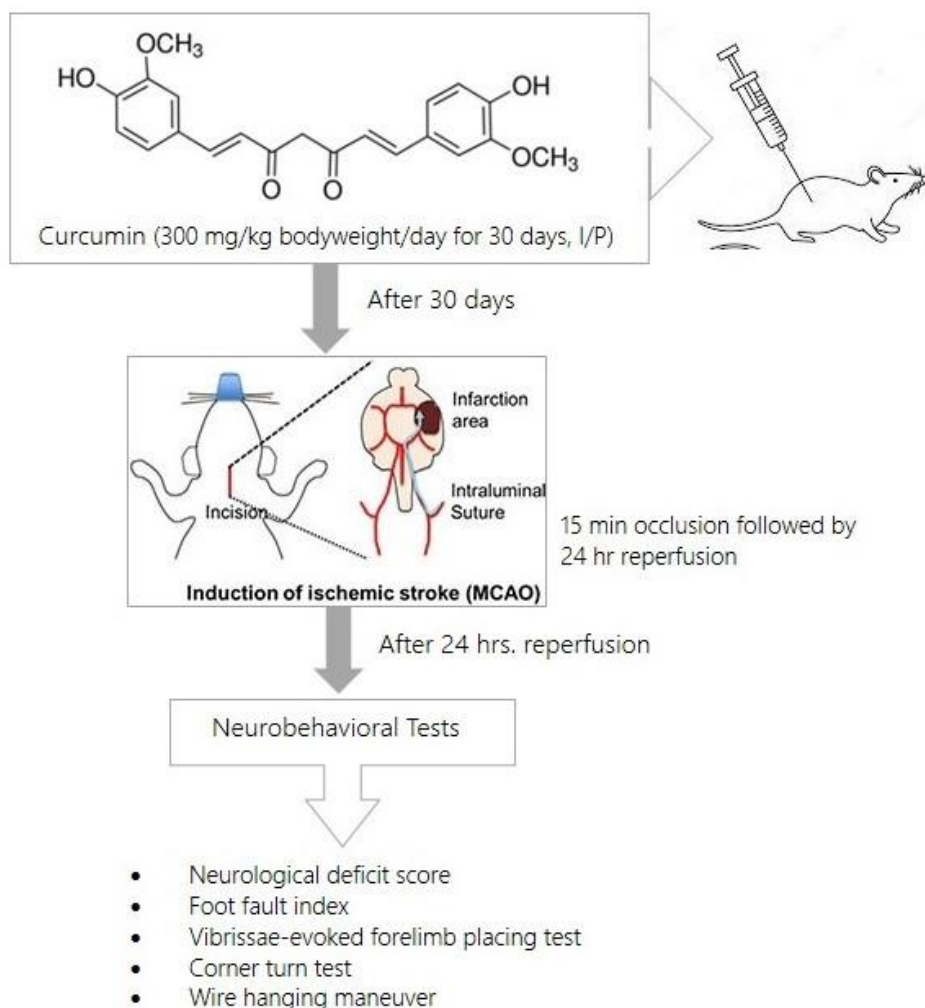


Figure 1: Schematic diagram showing the experimental protocol

Treatment Protocol

Curcumin dose was prepared according to the previously described method²². The MCAO+CUR group's animals receive curcumin 300 mg/kg body weight, dissolved in normal saline (pH 7.4), and administered intraperitoneally for 30 days. On the 31st day, animals were subjected to MCAO for 15 min, followed by reperfusion of 24 hrs.

MCAO Protocol

Transient MCAO was induced via the monofilament method as described in detail by previous studies^{23,24}.

Anesthesia

Animals were anesthetized intraperitoneally with a mixture of xylazine (5 mg/kg) and ketamine hydrochloride (50 mg/kg)²³. Anesthetic depth was confirmed with the absence of a paw-pinch reflex. Body temperature was maintained at $37.0 \pm 0.5^\circ\text{C}$, and other body conditions were monitored perioperatively.

MCAO surgery

For MCAO induction, left common, external, and internal carotid arteries were exposed after ventral midline cervical incision. Then a 4-0 monofilament silicon-coated suture with a rounded tip of 0.37 mm diameter was inserted at the bifurcation of the left common carotid artery and advanced until the monofilament occluded the left middle cerebral artery (MCA). After 15 minutes of occlusion, the monofilament was withdrawn to allow for reperfusion (24 hrs.). The neck skin incision was sutured back, and the animal was allowed to recover^{10,25}.

Sham surgery

Sham-operated animals receive the same MCAO surgery, including the exposure of all carotid arteries without monofilament insertion and occlusion of the middle cerebral artery. *Of note: control, sham and MCAO groups were based on previous literature²⁵.

Neurobehavioral Testings

At the end of the study protocol, all experimental groups were examined for MCAO-induced SNL

associated sensorimotor changes using a battery of different neurobehavioral tests.

Neurobehavioral tests used in this study include neurological deficit score, foot-fault test, vibrissae evoked forelimb placing test, corner turn test, and wire hanging test.

Procedures for Neurobehavioral Tests

All neurobehavioral testings were done by qualified persons blinded to experimental groups. To ensure accurate neurobehavioral results, animals were handled and trained for one week before the baseline testing. Also, all the training and testings were done in the animals' housing cage and in the light phase (approx. same time) daily.

Neurological Deficit Score

Neurological deficit (ND) score is a composite scoring used to reveal the ischemic stroke associated neurological deficits in animals²⁶. ND-score has been performed 24 hrs. post-MCAO. This neurological battery consists of the following three tests:

- (1) Body Twisting: test postural reflex
- (2) Forelimb Flexion: test sensorimotor integration
- (3) Balance Test: test limb dysfunction

The overall composite score is based on predetermined criteria²⁶. Total ND-score graded on a scale of 0 – 9. Where 0 represents normal, and 9 represents the maximum neurological deficit as described²⁶.

Foot Fault Test

The foot fault asymmetry test is commonly used to evaluate the post-stroke motor coordination and sensorimotor functioning and rehabilitation effects in MCAO experimental models¹³. The foot fault test is a skilled task in which animals were trained to walk on an elevated grid to record the number of foot faults (limb slip) as rats with ischemic brain damage exhibit greater paw-misplacements than normal rats²⁷ so the total number of foot-faults for forelimb and hindlimb were recorded as and scored as foot fault index (FFI).

$\text{FFI} = (\text{contralateral faults} - \text{ipsilateral faults}) \div (\text{total steps})$. The FFI rated on the scale as zero indicates

no asymmetry, the score of the negative number indicates ipsilateral deficit while the positive score represents contralateral deficit²⁷.

Vibrissae Evoked Forelimb Placing Test

This test allows us to study the focal cerebral ischemia associated with neurological dysfunction and the effect of treatment interventions on post-stroke rehabilitation²⁸. For this task, the animal was lifted by torso to freely hang in the air as its forelimbs and hindlimbs freely hung in the air. For the induction of ipsilateral and contralateral forelimb responses, the animal's vibrissae were brushed at the edge of a table. In response, animals (without any brain injury) place a forelimb ipsilateral to vibrissae-evoked side while MCAO animals with the motor deficit would exhibit impaired limb placement²⁸. Therefore, animals were tested for two sub-types of vibrissae-evoked forelimb placing test.

1. Same side / Ipsilesional forelimb placement.
2. Cross midline / Contralesional forelimb placement test.

Animals underwent 10 trials; each time percent successful limb-placement was recorded and computed as [% = (Paw placement ÷ 10) × 100]. Scoring less % represents sensorimotor impairment.

Corner Turn Test

Corner turn (CT) test, a well-known sensorimotor test to identify the functional deficits and sensory-motor (postural) asymmetries after ischemic stroke. This test examined the rodent's reliance on a preferential limb and so turned in a specific direction²⁹. In this test, the animal was placed and allowed to reach the corner and noted the turns (left or right) taken by the animal either by place one or both forelimbs. Animals with MCAO damage exhibit reliance on a non-impaired limb and turning in an ipsilateral direction. The animal underwent 10 trials, and a reliant turning side was

noted. Results scored as % = [(Number of ipsilateral (left) turns) ÷ (Total number of right turns + left turns) × 100]²⁹.

Wire Hanging Test

This is a simple test used to evaluate the post-MCAO motor functioning based on animal strength of grip, endurance, balance, and sensorimotor coordination. Animals with ischemic brain injury show poor performance in wire holding task and have very short latency of fall^{26,30}. In this task, the animal was allowed to hang on a wire, and the wire holding time (latency to fall) was noted^{30,31}. The increased wire holding time indicates better grip strength and muscle endurance of animals.

Statistical Analysis

Data were tabulated and expressed as mean ± standard error of the mean (SEM); values in percentage (%) are presented as mean ± standard deviation (SD). Statistical analysis was performed using statistical software SPSS 17.0. Intergroup differences were tested with ANOVA followed by post hoc t-test. P < 0.05 was considered statistically significant.

Results

Animals from all groups showed normal behavioral results before MCAO or sham surgery. Table 1 showed the comparison of neurobehavioral tests among different experimental groups.

Neurological Deficit Score

The MCAO group showed a significantly higher neurological deficit score (7.83 ± 0.47) as exhibited by impaired neurological performance compared to control and sham groups.

Rats treated with curcumin (MCAO+CUR) showed an improved ND score (5.66 ± 0.667) compared to the MCAO group (p < 0.05) (Table 1).

Table 1: Comparison of neurological deficit scores

Experimental Groups (n=9)	Neurological Deficit Score 24 hrs after reperfusion
Control	0
Sham	0.833±0.401
MCAO	7.833±0.477 ^{***/*}
MCAO+CUR	5.66±0.667 ^{***/*} /NS

Values are presented as mean ± SEM. Significance level *p < 0.05; **p < 0.01; *** p < 0.005, NS = non-significant, compared with control/compared with sham/compared with MCAO.

MCAO: middle cerebral artery occlusion; CUR: curcumin.

Foot-Fault Test

As shown in Table 2, animals from the control and sham groups showed no foot faults. MCAO group exhibit poor performance in foot fault task. MCAO group rats scored higher for hind limb than forelimb as exhibited by higher scores (FFI_{Hindlimb} = 0.15 ± 0.042; FFI_{Forelimb} = 0.1 ± 0.025), indicating that MCAO affects contralateral hindlimb more than contralateral forelimb.

Curcumin pre-treated group (MCAO+CUR) showed lower foot-faults (FFI_{Hindlimb} = 0.1 ± 0.025, FFI_{Forelimb} = 0.067 ± 0.021) compared to MCAO group but did not reach the statistical significance, p > 0.05 for both forelimb and hindlimb.

Table 2: Comparison of foot fault index

Experimental Groups (n=9)	FFI _{Forelimb}	FFI _{Hindlimb}
Control	0	0
Sham	0	0
MCAO	0.1±0.025	0.067±0.021 ^{NS/NS}
MCAO+CUR	0.15±0.042	0.1±0.025 ^{NS/NS}

Values are presented as mean ± SEM. Significance level *p < 0.05; **p < 0.01; *** p < 0.005, NS = non-significant, compared with control/compared with sham/compared with MCAO.

MCAO: middle cerebral artery occlusion; CUR: curcumin; FFI: foot-fault index

Vibrissae evoked Forelimb Placing Test

Among all other experimental groups, rats from the MCAO group showed a significantly higher deficit for the forelimb placement test. Also, the MCAO group scored significantly (p < 0.001) higher for contralateral forelimb than ipsilateral forelimb compared to the control group (Table 3).

While compared to MCAO rats, curcumin supplemented group MCAO+CUR scored significantly better in vibrissae-evoked forelimb placing test (% ipsilesional limb placement = 73.3 ± 0.816 %, p < 0.001) and (% contralesional limb placement = 53.3 ± 1.96 %, p < 0.01).

Table 3: Comparison of ipsilesional and contralesional limbs placement in vibrissae evoked forelimb placing test

Experimental Groups (n = 9)	Vibrissae Evoked Forelimb Placing Test	
	Ipsilesional Limb Placement (%)	Contralesional Limb Placement (%)
Control	91.70±0.408	91.70±0.752
Sham	80±1.095*	88.30±0.752 ^{NS}
MCAO	21.70±0.752 ^{***/**}	13.30±1.21 ^{***/**}
MCAO+CUR	73.3±0.816 ^{***/NS/**/NS}	53.3±1.966 ^{***/**/NS}

Values are presented as Mean (%) ± SD. Significance level *p < 0.05; **p < 0.01; *** p < 0.005, NS = non-significant, compared with control/compared with sham/compared with MCAO.
MCAO: middle cerebral artery occlusion; CUR: curcumin

Corner Turn Test

As displayed in Table 4, animals from the control group did not show corner turn asymmetries as exhibited by approx. equal number of left and right turns (average left turns 53.3 ± 0.852 % and right turns 46.7 ± 0.816 %). Similarly, sham-operated rats also did not exhibit any significant limb reliance. The MCAO group showed significantly higher percentage of ipsilateral (left) turns (71.66 ± 0.752 %) as compared to control (p < 0.01) and sham-operated (p < 0.01) groups exhibiting corner turn asymmetry and reliance on non-impaired (left) limb. In MCAO+CUR treated group; decreased % ipsilateral turns (66.66 ± 1.211 %) were noted as compared to MCAO group (p > 0.05).

Table 4: Comparison of percent left (ipsilesional) turns in corner turn test

Experimental Groups (n = 9)	Corner Turn Test
	(% Ipsilateral turns)
Control	53.33±0.852
Sham	48.33±1.169 ^{NS}
MCAO	71.66±0.752 ^{**/**}
MCAO+CUR	66.66±1.211 ^{NS*/NS/NS}

Values are presented as Mean (%) ± SD. Significance level *p < 0.05; **p < 0.01; *** p < 0.005, NS = non-significant, compared with control/compared with sham/compared with MCAO.
MCAO: middle cerebral artery occlusion; CUR: curcumin.

Wire Hanging Test

Compared to the control and sham-operated groups, animals of the MCAO group exhibit poor wire holding as displayed by significantly decreased wire-holding time (15.5 seconds) (p < 0.001 for control vs. MCAO) and (p < 0.001 for sham vs. MCAO) groups (Table 5). Curcumin pre-treated animals (MCAO+CUR) showed significantly better wire holding time (23.5 seconds) when compared to the MCAO untreated group (p < 0.01).

Table 5: Comparison of wire holding time (seconds)

Experimental Groups (n = 9)	Wire Hanging Maneuver
	(Latency to fall in seconds)
Control	57.66±0.61
Sham	52.66±0.66*
MCAO	15.5±1.52 ^{***/**}
MCAO+CUR	23.5±1.94 ^{***/**/NS}

Values are presented as mean ± SEM. Significance level *p < 0.05; **p < 0.01; *** p < 0.005, NS = non-significant, compared with control/compared with sham/compared with MCAO.
MCAO: middle cerebral artery occlusion; CUR: curcumin.

Discussion

Globally, stroke is the single major cause of disabilities affecting around 15 million people⁷. Ischemia-reperfusion mediated oxidative stress initiates the myriad of pathological processes that ultimately lead to neuronal death and tissue necrosis⁹. Due to these catastrophic cellular events, patients suffer devastating sensorimotor and cognitive impairments after ischemic stroke³². Furthermore, very limited options are available to treat ischemic stroke survivors. Hence identification of an effective management approach, including treatment with preventive measures is a challenging and urgent need³. Herbs and natural products have been long recognized for neuroprotective potentials mainly attributable to their antioxidant and anti-inflammatory effects⁵. Therefore, the present study aimed for functional assessments in the MCAO rats under curcumin pre-treatment. MCAO-induced regional cerebral injuries exhibited by sensorimotor dysfunction. This study includes various behavioral testings to evaluate the transient ischemia associated with neurological dysfunction and the treatment regime's effect (300 mg curcumin/kg bodyweight for 30 days) in MCAO induced rats.

Results of the present study also displayed the functional deficits in the MCAO group as exhibited by significantly higher ND score ($p < 0.005$), high foot fault index, poor grip and memory, impaired forelimb placing ($p < 0.005$), wire-hanging ($p < 0.005$) and corner turn ($p < 0.01$) tests when compared with normal animals. The previous studies^{6,33} also reported the MCAO associated sensory, motor, and cognitive deficits principally because of ROS-mediated apoptosis and necrosis cascade and neuroinflammation.

30- days curcumin pre-treatment at a dose of 300 mg/kg body weight found to effectively prevent a sensorimotor deficit in animals of MCAO+CUR group when compared with the MCAO group as shown by improved ND-score, better score in corner turn test (less ipsilesional turns). The MCAO+CUR treated group displayed increased endurance, grip strength, wire-holding time, and fewer foot-faults than the MCAO group ($p < 0.005$),

highlighting the neuroprotective potentials of curcumin against transient cerebral ischemic insult.

In line with our study results, various other experimental studies on MCAO rats also reported improved sensorimotor and cognitive functioning with curcumin treatment^{17,34}. According to previous research studies, curcumin mediated neuroprotective effects are attributable to its antioxidant, anti-inflammatory, and anti-apoptotic properties^{17,19,35,36}.

A study by Liu et al. displayed decreased infarct size, improved neurological functions, and higher PPAR γ (peroxisome proliferator-activated receptor-gamma) expression in MCAO rats under curcumin treatment compared to MCAO untreated group. Liu et al. demonstrated the molecular targets of curcumin and concluded that curcuminoids administration significantly upregulates the PPAR γ in MCAO induced rats. PPAR γ is an anti-inflammatory transcription factor hence mediate neuroprotection against ischemia-reperfusion injury³⁷.

Another experimental study also reported that curcuminoid pre-treatment significantly reduces cerebral edema and the size of ischemic infarct in the rat model of transient-MCAO³⁸. Curcumin supplementation significantly reverses the post-stroke oxidative stress and neurological dysfunctions as exhibited by improved neurobehavioral tests, attributed to curcumin antioxidant potentials³⁸. Antioxidant potentials of curcumin include a potent scavenger of free radicals, increases endogenous antioxidants enzymes, and modulates γ -enolase pathways^{19,35}.

Conclusion

Taken together, the data of the present study indicate that transient ischemic stroke results in a selective neuronal loss that impaired neurological functions. Designed curcumin treatment regime found to be effective against the sensory, motor, and cognitive functions in the MCAO rat model of ischemic stroke. Curcumin mediated neuron survival principally by managing oxidative tissue damage.

Conflicts of Interest

None.

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