

The effect of intravenous administration of liposomal curcumin in addition to sumatriptan treatment in an experimental migraine model in rats

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Background: Curcumin has antioxidative properties that could be useful in various diseases due to its ability to act on multiple targets of various cellular pathways. We aimed to assess the efficacy of liposomal curcumin compared with curcumin solution, when in addition to sumatriptan (ST) treatment, in an experimental migraine model induced with nitroglycerin (NTG) in rats.

Methods: Seven groups of 9 rats each were investigated: control group without migraine (1 mL saline solution intraperitoneal injection [ip]), control group with induced migraine, NTG+ST group (ST), NTG+ST+curcumin1 (CC1) group – 1 mg/100 g body weight (bw), NTG+ST+CC2 – 2 mg/100 g bw, NTG+ST+liposomal curcumin1 (ICC1) group – 1 mg/100 g bw, and NTG+ST+ICC2 (ICC2) group – 2 mg/100 g bw. NTG and ST were administered as 1 mL ip NTG | 1 mg/100 g bw and 1 mL ip ST | 1 mg/100 g bw, respectively. Plasma total oxidative stress (TOS), malondialdehyde (MDA), nitric oxide (NOx), thiol levels, as well as total antioxidative capacity (TAC) were assessed. The nociception process was assessed by counting the number of flinches and shakes after the formalin test.

Results: The plasma TOS, MDA, and NOx levels, as oxidative stress parameters, were significantly decreased in the curcumin-treated groups, especially where curcumin was in liposomal form. The thiol and TAC were also improved by the curcumin treatment, with the best results obtained for the liposomal curcumin. The closest number of flinches and shakes to the control group was obtained for the group treated with liposomal curcumin at a dose of 2 mg/100 g bw.

Conclusion: Liposomal curcumin in a dose of 2 mg/100 g bw when in addition to ST treatment could be an optimum therapeutic strategy for migraine attacks and could represent a base for future clinical research and application.

Keywords: rats, migraine disorder, liposomal curcumin, serotonin, oxidative stress, nociception

Introduction

The Global Burden of Disease Study reported tension-type headache and migraine as the second most prevalent disorders in the world.¹ There is still insufficient knowledge about the mechanisms of migraine, and different treatment strategies are currently under research (both preventive therapy and acute therapy for migraine attack). The neurogenic vasodilation is one of the most important mechanisms of migraine due to an excessive production of nitric oxide (NO) that activates a long-lasting central mechanism of hyperalgesia.² Consequently, the experimental migraine induced by nitroglycerin (NTG) administration (an NO donor) is one of the most reliable experimental models for migraine inducement in rats.³ NTG-induced hyperalgesia can be evaluated by assessment of the

nociceptive behavior evoked by the formalin test.² Common migraine triggers are capable of generating oxidative stress, and oxidative-stress molecules released during migraine attack can contribute to nociceptive mechanism.^{4,5} The nitro-oxidative stress and the inflammatory response play a major role in the onset and development of neuropathic pain, and antioxidant supplementation therapy seems to be useful.⁶ Considering these pieces of evidence assessing the oxidative stress during migraine attack could be useful for nociception evaluation as well as for establishing the treatment's efficiency.

The preemptive analgesic and antioxidant effects of curcumin following oral administration in an experimental migraine model induced by NTG in rats have been previously demonstrated.^{7,8} Because the bioavailability of curcumin administered orally is poor, primarily due to poor absorption, rapid metabolism, and fast elimination,⁹ in our study we opted for the parenteral administration of curcumin. Following parenteral administration, namely the intravenous route, curcumin nanoformulation (such as curcumin-loaded liposomes and polymeric nanoparticles obtained from acrylic polymers or poly(lactic-co-glycolic acid)), has been shown to reach the central nervous system parenchyma by crossing the blood–brain barrier (BBB).¹⁰ Curcumin solutions were also administered by intravenous route for its anti-inflammatory properties in adjuvant-induced arthritis in rats.¹¹ In a clinical trial, nanocurcumin plus omega-3 fatty acids supplementation have been shown to reduce both the attack frequency and the ICAM-1 (adhesion molecule associated with inflammation) serum level in patients with migraine.¹² Therefore, nanoformulations of curcumin have been developed for their superior therapeutic efficiency in inflammatory and oxidative stress processes.^{13,14}

The serotonergic system represents one of the most important pathophysiological mechanisms of migraine; 5-hydroxytryptamine₁ (5-HT₁) receptor agonists are known to be effective in the treatment of migraine attacks.¹⁵ The 5-HT₁ receptors are subdivided into 5-HT_{1A–D}, and the effect of triptans is related to 5-HT_{1B/1D} receptor occupancy.¹⁶ Consequently, one of the pathological mechanisms of migraine attack is related to the activation of 5-HT₁ receptors in the brain and their genotype.¹⁵ The plasmatic level of 5-HT decreases, and the concentration of its metabolite, hydroxyindoleacetic acid, increases between attacks and decreases during the attack.¹⁷ A low concentration of 5-HT facilitates the activation of the trigeminovascular pathway induced by cortical spreading depression.¹⁸ It was also demonstrated that about a half of the rat trigeminal ganglion neurons contain nitric oxide.¹⁹ Depletion of serotonin, a vasoconstrictor, would leave the vasodilator nitric oxide to trigger

the migraine attack in experimental migraine in rats.²⁰ One of the most effective drugs for migraine attack is sumatriptan (ST), a selective 5-HT₁ receptor agonist. Additionally, ST can also produce a transient increase in systolic and diastolic blood pressure when it is administered by intravenous route.²¹ Even though there are several limitations of triptan treatment (serotonin syndrome with symptoms that include agitation, diarrhea, nausea, and loss of coordination),²² ST remains one of the most widely used treatments for migraine attack.²¹ The antioxidant effect of curcumin (including the reduction of nitro-oxidative stress and NO plasma concentration during experimental migraine) and its analgesic properties have been previously demonstrated.^{7,8} Our study aimed to compare the effect of single ST administration with the association of ST and curcumin for the treatment of migraine attacks in an experimental migraine model induced with NTG in rats and to observe if there is a synergistic effect of ST and curcumin on oxidative stress. Because the absorption of curcumin after oral administration is limited, our experiment was designed to observe the effect of curcumin-loaded liposomes following intravenous administration, in addition to ST treatment.

Materials and methods

Materials

ST (Imigran) was purchased from GlaxoSmithKline PLC (London, UK). Curcumin ($\geq 94\%$ curcuminoid content; $\geq 80\%$ curcumin) and cholesterol were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC, $\geq 99\%$ [TP-PC]) and N-(carbonyl-methoxypolyethylenglycol-2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (PEG-2000-DSPE, $\geq 98\%$ [HPLC]) sodium salt were purchased from Lipoid GmbH (Ludwigshafen, Germany). All other chemicals were of analytical grade.

Experimental model

This experimental study used Wistar-Bratislava albino male rats (from the Animal Department of the University of Medicine and Pharmacy in Cluj-Napoca), weighing 200–250 g. The animals were kept at the Pathophysiology Department in polypropylene cages at constant temperature ($24^{\circ}\text{C} \pm 2^{\circ}\text{C}$), humidity ($60\% \pm 5\%$), and light-dark regime. The animals had free access to standard pellets (Cantacuzino Institute, Bucharest, Romania) and to water ad libitum.

The rats were randomly divided in 7 groups containing 9 animals/group: one control group without migraine (C), one control group with induced migraine (NTG-C), one group with ST only, two groups with ST and different

doses of curcumin solution (NTG+ST+curcumin1 [CC1] and NTG+ST+CC2), and two groups treated with ST and different doses of liposomal curcumin (NTG+ST+liposomal curcumin1 [ICC1] and NTG+ST+ICC2), as presented in Table 1.

Preparation of liposomal curcumin

Liposomal curcumin was prepared as PEG-coated nano-liposomes using the film hydration method, as previously described.²³ Briefly, the phospholipids were used in a concentration of 70 mM, of which 66.5 mM DPPC, the main lipid, and 3.5 mM PEG-2000-DSPE, a PEG-derived phospholipid, to achieve long circulation properties and an increased therapeutic efficiency. Cholesterol was added to the lipid mixture in 10:1 phospholipids to cholesterol molar ratio. Liposomal curcumin was characterized for mean particle size, polydispersity value, zeta potential, curcumin content, and encapsulation efficiency.^{22,24} Thus, liposomal curcumin had a size around 140 nm; a low polydispersity value, close to 0.1; and a zeta potential of about -50 mV. The size, size distribution, and surface charge of the liposomes were determined immediately after preparation, upon dilution in a ratio of 1:100. The presented method led to an efficient encapsulation of curcumin in the liposomes, the encapsulation efficiency being close to 80%, and so the formulation contained a relatively high concentration of curcumin, around 4.7 mg/mL. On the other hand, a curcumin solution was prepared by dissolution in 96% (v/v) ethanol and was further diluted with saline to achieve the same curcumin concentration as in the liposomal formulation.

ST and curcumin administration

In the NTG+ST group, ST at a dose of 1 mg/kg body weight (bw) was injected ip 30 min after the NTG injection, in accordance with previous studies.^{25,26} Curcumin was administered intravenous administration immediately after ST, in the dorsal vein of the tail, according to Chiu et al.¹⁰

Formalin test

The formalin test, for nociception assessment, was performed 4 h after the NTG administration⁸ and was carried out as described by Tjølsen et al.²⁷ Diluted formalin (1% in saline) was injected subcutaneously in the right paw of the animals. After the formalin injection, animals were kept in plexiglass cages for observation. Two distinct phases were assessed. The number of flinches and shakes (rapid and brief withdraws or flexions of the injected paw) per min were assessed during 1–5 min after the formalin injection (Phase I) and for 1-min periods at 5-min intervals during 10–60 min after the formalin injection (Phase II). The vasodilator effect consecutive to a noxious stimulus is characteristic of the first phase, whereas the inflammatory mechanism induced by noxious stimuli is the central phenomenon in the second phase.²⁸

Measurements

The oxidative stress parameters were quantified from blood samples collected from the retro-orbital plexus of each rat, under ketamine anesthesia (5 mg/kg bw, ip route).²⁹ The animals were euthanized at the end of the experiment by cervical dislocation. The following oxidative stress parameters were assessed:

Table 1 Groups by inducement and treatment regimens

Group	Abbreviation	Administration route dose
1 (control)	C	1 mL ip saline solution (0.9%)
2 (NTG control)	NTG-C	1 mL ip NTG 1 mg/100 g bw
3 (NTG+ST)	NTG+ST	1 mL ip NTG 1 mg/100 g bw 1 mL ip ST 1 mg/100 g bw
4 (NTG+ST+curcumin1)	NTG+ST+CC1	1 mL ip NTG 1 mg/100 g bw 1 mL ip ST 1 mg/100 g bw 1 mL iv curcumin solution 1 mg/100 g bw
5 (NTG+ST+curcumin2)	NTG+ST+CC2	1 mL ip NTG 1 mg/100 g bw 1 mL ip ST 1 mg/100 g bw 1 mL iv curcumin solution 2 mg/100 g bw
6 (NTG+ST+liposomal curcumin1)	NTG+ST+ICC	1 mL ip NTG 1 mg/100 g bw 1 mL ip ST 1 mg/100 g bw 1 mL iv liposomal curcumin 1 mg/100 g bw
7 (NTG+ST+liposomal curcumin2)	NTG+ST+ICC2	1 mL ip NTG 1 mg/100 g bw 1 mL ip ST 1 mg/100 g bw 1 mL iv liposomal curcumin 2 mg/100 g bw

Abbreviations: bw, body weight; CC, curcumin; ip, intraperitoneal injection; iv, intravenous administration; ICC, liposomal curcumin; NTG, nitroglycerin; ST, sumatriptan.

- oxidative stress intensity: malondialdehyde (MDA),³⁰ the indirect assessment of nitric oxide (NOx) synthesis, and total oxidative status (TOS)³¹
- the antioxidant capacity of the plasma: thiol³² and total antioxidative capacity (TAC)³⁰

A Jasco V-350 UV-VIS spectrophotometer (Jasco International Co, Ltd, Tokyo, Japan) was used for all spectroscopic measurements.

Statistical analysis

The data were analyzed using Statistica program (v.8, StatSoft) and presented as mean±SD, and as median and interquartile ranges (expressed as [Q1–Q3], where Q1 and Q3 are the value of the first and third quartiles). Statistical comparisons between groups were performed using the two-tailed Mann–Whitney test at a significance level of 5%. All graphs were plotted using Microsoft Excel 2016.

Ethics statement

The Ethics Committee of Iuliu-Hațieganu University of Medicine and Pharmacy Cluj-Napoca (454/20.12.2017) approved the study. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Results

The parameters that reflect the oxidative stress intensity varied as expected among groups. The lowest values were obtained for the group treated with liposomal curcumin at a dose of 2 mg/100 g bw. For different groups treated with curcumin, the highest mean values of the oxidative stress parameters were observed in the NTG+ST+CC1 group for MDA and in the NTG+ST+CC2 group for NOx and TOS. The lowest values for the parameters related to the antioxidant capacity of plasma were observed for the NTG+ST group. The highest concentration of thiol and TAC were found in the NTG+ST+CC2 group. The statistical summary of the oxidative stress parameters is presented in Tables 2 (oxidative stress intensity parameters) and 3 (antioxidant capacity of plasma).

The MDA values were significantly different compared to control for all groups treated with curcumin, either conventional or liposomal ($p < 0.0004$). The smallest variability in MDA values was observed for the NTG+ST+CC1 group, with significant differences in all groups treated with curcumin compared to the control group (C), NTG group, or NTG+ST group (Figure 1A). Such as the MDA values, the NOx values were significantly different compared to control for all groups that received curcumin, either conventional or liposomal ($p < 0.0004$). No significant differences were observed between the groups treated with the same type of curcumin but at different concentrations (conventional

Table 2 Quantification of oxidative stress intensity by groups

Group	Statistics	MDA (pmol/L)	NOx (μmol/L)	TOS (μmol/L)
C	Mean±SD	2.59±0.06	14.89±2.03	26.56±1.67
	Median (Q1–Q3)	2.61 (2.55–2.62)	15 (14–17)	26 (25–27)
NTG-C	Mean±SD	4.66±0.13	37.89±2.67	46.00±2.24
	Median (Q1–Q3)	4.66 (4.56–4.75)	37 (36–40)	46 (45–46)
NTG+ST	Mean±SD	4.33±0.09	35.00±3.54	36.78±3.99
	Median (Q1–Q3)	4.33 (4.29–4.4)	35 (31–38)	36 (35–40)
NTG+ST+CC1	Mean±SD	4.20±0.02	32.22±2.86	33.56±3.68
	Median (Q1–Q3)	4.2 (4.19–4.21) ^{a,e,i}	33 (30–35) ^{A,E}	35 (31–36) ^{*1,#4}
NTG+ST+CC2	Mean±SD	3.95±0.40	32.78±1.30	35.89±1.76
	Median (Q1–Q3)	4 (3.9–4.2) ^{b,f,j,m}	33 (32–33) ^{B,F}	36 (35–37) ^{*2,#5}
NTG+ST+CC1	Mean±SD	3.51±0.23	23.22±1.99	26.78±2.73
	Median (Q1–Q3)	3.5 (3.39–3.67) ^{c,g,k}	23 (22–25) ^{C,G,I}	27 (26–29) ^{#6,#8}
NTG+ST+CC2	Mean±SD	3.11±0.24	21.33±2.18	22.67±1.80
	Median (Q1–Q3)	3.12 (2.89–3.23) ^{d,h,l,n}	21 (20–23) ^{D,H,J}	23 (21–24) ^{*3,#7,#9,±10}

Notes: Comparisons (Mann–Whitney test): p -values compared with C group: MDA comparisons: ^a3.39×10⁻⁴, ^b3.44×10⁻⁴, ^c3.46×10⁻⁴, ^d3.46×10⁻⁴; NOx comparisons: ^A3.29×10⁻⁴, ^B3.09×10⁻⁴, ^C3.29×10⁻⁴, ^D3.29×10⁻⁴; TOS comparisons: ^{*1}1.19×10⁻³, ^{*2}3.27×10⁻⁴, ^{*3}7.99×10⁻⁴; p -values compared with NTG-C group: MDA comparisons: ^e3.39×10⁻⁴, ^f4.71×10⁻⁴, ^g3.46×10⁻⁴, ^h3.46×10⁻⁴; NOx comparisons: [†]1.58×10⁻³, [‡]4.36×10⁻⁴, [§]3.39×10⁻⁴, [¶]3.39×10⁻⁴; TOS comparisons: ^{#4}3.20×10⁻⁴, ^{#5}3.18×10⁻⁴, ^{#6}3.20×10⁻⁴, ^{#7}3.18×10⁻⁴; p -values compared with NTG+ST group: MDA comparisons: [‡]8.95×10⁻⁴, [§]5.39×10⁻³, [¶]3.49×10⁻⁴, [‡]3.49×10⁻⁴; NOx comparisons: [‡]3.34×10⁻⁴, [‡]3.34×10⁻⁴; TOS comparisons: ^{#6}3.32×10⁻⁴; p -values compared with NTG+ST+CC1 group: MDA comparisons: [‡]4.62×10⁻²; p -value compared with NTG+ST+CC1: MDA comparisons: [‡]6.17×10⁻³; TOS comparisons: [‡]5.96×10⁻³.

Abbreviations: C, control; CC, curcumin; ICC, liposomal curcumin; MDA, malondialdehyde; NOx, nitric oxide; NTG, nitroglycerin; Q1, 25th percentile; Q3, 75th percentile; ST, sumatriptan; TOS, total oxidative status.

Table 3 Quantification of antioxidant capacity by groups

Group	Statistics	Thiol (mg%)	TAC (Eq/L)
C	Mean±SD	208.00±3.84	1.23±0.03
	Median (Q1–Q3)	207 (207–210)	1.23 (1.2–1.24)
NTG-C	Mean±SD	182.56±5.61	1.08±0.04
	Median (Q1–Q3)	183 (178–187)	1.07 (1.06–1.1)
NTG+ST	Mean±SD	185.78±7.19	1.12±0.06
	Median (Q1–Q3)	185 (179–189)	1.12 (1.11–1.16)
NTG+ST+CC1	Mean±SD	189.33±5.20	1.16±0.30
	Median (Q1–Q3)	191 (189–192) ^{a,e}	1.08 (1.04–1.09) ^A
NTG+ST+CC2	Mean±SD	193.67±5.10	1.37±0.18
	Median (Q1–Q3)	195 (190–198) ^{b,f,i}	1.37 (1.21–1.45) ^{D,G}
NTG+ST+ICC1	Mean±SD	217.56±4.28	1.44±0.05
	Median (Q1–Q3)	218 (213–222) ^{c,g,j}	1.44 (1.4–1.47) ^{B,E,H,J}
NTG+ST+ICC2	Mean±SD	225.56±4.69	1.55±0.09
	Median (Q1–Q3)	226 (223–229) ^{d,h,k,l}	1.58 (1.51–1.61) ^{C,F,I,K}

Notes: Comparisons (Mann–Whitney test): *p*-values compared with C group: thiol comparisons: ^a3.34×10⁻⁴, ^b3.34×10⁻⁴, ^c8.95×10⁻⁴, ^d3.39×10⁻⁴; TAC comparisons: ^A5.29×10⁻³, ^B3.44×10⁻⁴, ^C3.44×10⁻⁴; *p*-values compared with NTG-C group: thiol comparisons: ^e1.46×10⁻², ^f1.65×10⁻³, ^g3.39×10⁻⁴, ^h3.44×10⁻⁴; TAC comparisons: ^D3.41×10⁻⁴, ^E3.41×10⁻⁴, ^F3.41×10⁻⁴; *p*-values compared with NTG+ST group: thiol comparisons: ⁱ2.13×10⁻², ^j3.44×10⁻⁴, ^k3.49×10⁻⁴; TAC comparisons: ^G3.41×10⁻⁴, ^H3.41×10⁻⁴; *p*-value compared with NTG+ST+CC1 group: ^l3.41×10⁻⁴; *p*-value compared with NTG+ST+ICC1: thiol comparisons: ^a4.65×10⁻³; TAC comparisons: ^I5.29×10⁻³, ^J1.31×10⁻².

Abbreviations: C, control; CC, curcumin; ICC, liposomal curcumin; NTG, nitroglycerin; Q1, 25th percentile; Q3, 75th percentile; ST, sumatriptan; TAC, total antioxidative capacity.

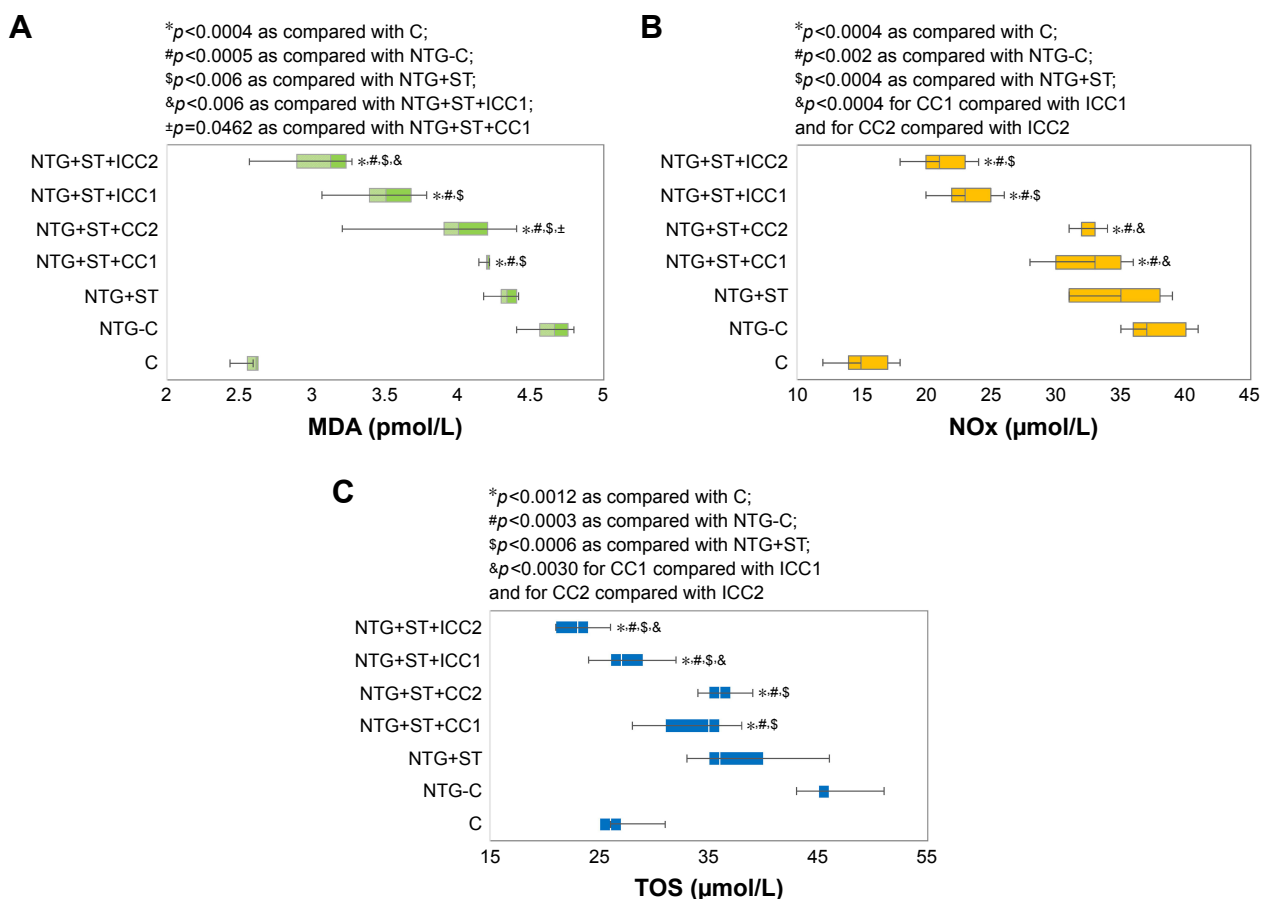


Figure 1 Parameters of oxidative stress intensity by groups. **(A)** MDA, **(B)** NOx, **(C)** TOS. The box is given by the values of the first and third quartiles, the mid-line is given by the value of the median, and the whiskers are the minimum and maximum values. C is the control group (no medication), NTG-C is the group treated with nitroglycerin (NTG), NTG+ST is the group treated with NTG and sumatriptan (ST), NTG+ST+CC1 is the group treated with NTG+ST and curcumin 1 mg/100 g body weight (bw), NTG+ST+CC2 is the group treated with NTG+ST and curcumin 2 mg/100 g bw, NTG+ST+ICC is the group treated with NTG+ST and liposomal curcumin 1 mg/100 g bw, and NTG+ST+ICC2 is the group treated with NTG+ST and liposomal curcumin 2 mg/100 g bw.

Abbreviations: C, control; MDA, malondialdehyde; NOx, nitric oxide; TOS, total oxidative status.

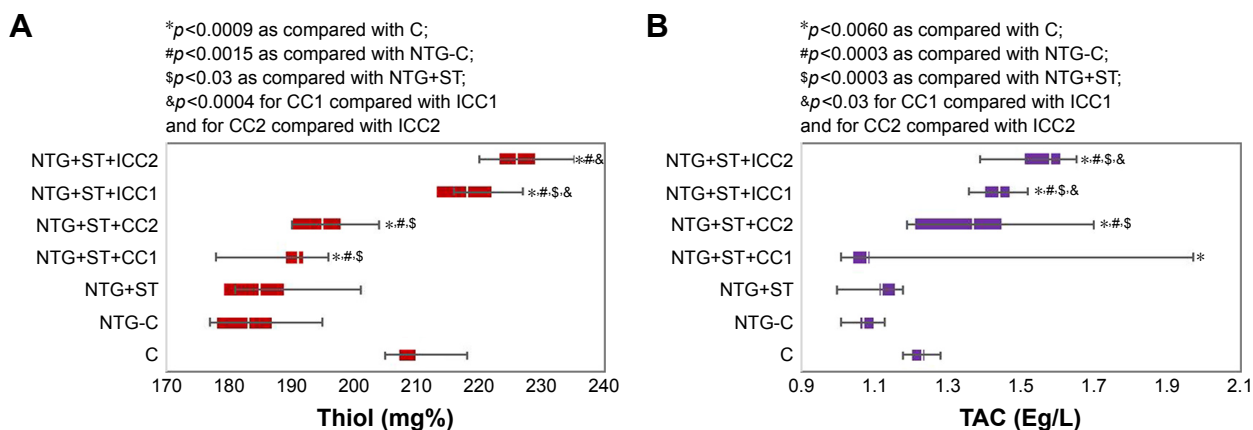


Figure 2 Parameters of antioxidant capacity of plasma by groups: **(A)** thiol, **(B)** TAC. The box is given by the values of the first and third quartiles, the mid-line is given by the value of the median, and the whiskers are the minimum and maximum values. C is the control group (no medication), NTG-C is the group treated with nitroglycerin (NTG), NTG+ST is the group treated with NTG and sumatriptan (ST), NTG+ST+CC1 is the group treated with NTG+ST and curcumin 1 mg/100 g body weight (bw), NTG+ST+CC2 is the group treated with NTG+ST and curcumin 2 mg/100 g bw, NTG+ST+ICC is the group treated with NTG+ST and liposomal curcumin 1 mg/100 g bw, and NTG+ST+ICC2 is the group treated with NTG+ST and liposomal curcumin 2 mg/100 g bw.

Abbreviations: C, control; TAC, total antioxidative capacity.

curcumin $p=0.75$, liposomal curcumin $p=0.08$). Significantly lower NOx values were found when the ICC2 group was compared to the CC2 group, and the ICC1 group was compared to the CC1 group (Figure 1B). Except for the NTG+ST+ICC1 group, the TOS values were significantly different compared to the control group (Figure 1C). The TOS values were significantly lower when the NTG+ST group was compared to NTG group ($p=0.0003$). No differences in the TOS concentration were seen when the CC1 group was compared to the CC2 group ($p=0.18$). The increase in liposomal curcumin dose led to a significant decrease of TOS values ($p=0.01$).

When compared to the control group, significant values were found for thiol ($p < 0.0009$), with higher values for the groups treated with liposomal curcumin and smaller values for all other groups (Figure 2A). No significant differences in thiol values were observed when NTG was compared with NTG+ST group ($p=0.38$). Similarly, thiol values were obtained among groups treated with conventional curcumin ($p=0.18$), while the increase in curcumin dose, in liposomal form, led to a significant increase in thiol values ($p=0.005$).

Except for the NTG+ST+CC2, the TAC values were significantly different compared to the control group. The TAC values were significantly higher in NTG+ST group compared to the NTG group ($p=0.03$). Significantly lower values of TAC were observed at a lower concentration of both conventional curcumin ($p=0.005$) and liposomal curcumin ($p=0.01$). The TAC values were significantly lower when the pretreatment consisted of conventional curcumin

as opposed to the group treated with liposomal curcumin (Figure 2B).

The smallest number of flinches and shakes in both phases (I and II) were observed for the NTG+ST+ICC2 group (16 in Phase I and 111 in Phase II), whereas the highest was found for the NTG group. The summary of the results obtained in the formalin test is presented in Table 4.

The number of flinches and shakes were significantly different when the pretreatment groups were compared with the control group, with one exception represented by the NTG+ST+ICC2 group (Figure 3). No significant differences with regard to the number of flinches and shakes were observed in the first phase when the NTG+ST+CC1 group was compared to the NTG+ST+CC2 group ($p=0.89$). In the second phase, the number of flinches and shakes were significantly lower in the group treated with the higher dose of conventional curcumin compared to the group that received the lowest dose of conventional curcumin ($p=0.01$). The number of flinches and shakes were significantly lower in both Phase I ($p=0.03$) and Phase II ($p=0.0003$) when the group treated with the higher dose of liposomal curcumin was compared to the group with the lowest dose of liposomal curcumin.

Discussion

NTG-induced hyperalgesia in rats is an experimental model that mimics the action of nitric oxide on amino acid neurotransmitters such as glutamate, which is a signaling molecule for central sensitization.³³ NO can cause migraine-like attacks in migraine patients and initiates a self-amplifying process in the trigeminal system, probably due to a central

Table 4 Descriptive metrics for the formalin test by groups

Group	Statistics	No of flinches and shakes	
		Phase I	Phase II
C	Mean±SD	20.11±1.76	114.11±2.93
	Median (Q1–Q3)	20 (19–21)	114 (113–117)
NTG-C	Mean±SD	42.22±2.68	146.44±9.86
	Median (Q1–Q3)	42 (41–44) ^a	145 (142–150) ^A
NTG+ST	Mean±SD	27.44±1.33	139.89±4.01
	Median (Q1–Q3)	28 (27–28) ^{b,f}	140 (137–144) ^B
NTG+ST+CC1	Mean±SD	27.78±4.41	136.78±4.97
	Median (Q1–Q3)	28 (26–32) ^{c,g}	138 (133–140) ^{C,F}
NTG+ST+CC2	Mean±SD	27.78±2.73	128.78±5.31
	Median (Q1–Q3)	28 (25–30) ^{d,h}	129 (125–131) ^{D,G,J,M}
NTG+ST+ICC1	Mean±SD	22.44±1.88	126.22±4.38
	Median (Q1–Q3)	22 (21–24) ^{e,i,k}	127 (122–129) ^{E,H,K}
NTG+ST+ICC2	Mean±SD	19.67±2.55	115.89±3.18
	Median (Q1–Q3)	20 (18–21) ^{j,l,m}	117 (113–118) ^{I,L,N}

Notes: Comparisons (Mann–Whitney test): *p*-values as compared with C group: Phase I: ^a3.32×10⁻⁴, ^b3.27×10⁻⁴, ^c1.40×10⁻³, ^d3.36×10⁻⁴, ^e3.39×10⁻⁴; Phase II: ^A3.41×10⁻⁴, ^B3.39×10⁻⁴, ^C3.39×10⁻⁴, ^D3.39×10⁻⁴, ^E3.39×10⁻⁴; *p*-values as compared with NTG group: Phase I: ^f3.25×10⁻⁴, ^g3.34×10⁻⁴, ^h3.34×10⁻⁴, ⁱ3.27×10⁻⁴, ^j3.32×10⁻⁴; Phase II: ^k1.33×10⁻², ^l1.07×10⁻³, ^m3.46×10⁻⁴, ⁿ3.46×10⁻⁴; *p*-values as compared with NTG+ST group: Phase I: ^o4.42×10⁻⁴, ^p3.27×10⁻⁴; Phase II: ^q1.45×10⁻³, ^r3.44×10⁻⁴, ^s3.44×10⁻⁴; *p*-value as compared with NTG+ST+CC1 group: Phase II: ^t7.88×10⁻³; *p*-value as compared with NTG+ST+ICC1: Phase I: ^u3.16×10⁻²; Phase II: ^v3.44×10⁻⁴.

Abbreviations: C, control; CC, curcumin; ICC, liposomal curcumin; NTG, nitroglycerin; Q1, 25th percentile; Q3, 75th percentile; ST, sumatriptan.

sensitization process.³⁴ Oxidative stress is also involved in central sensitization.³⁵ It has been shown that the administration of glutamate induces an increase in intracellular reactive oxygen species in cultured neurons.³⁶ The sensitization process refers to increased responsiveness to thermal, mechanical, or chemical stimuli, which may be translated to corresponding hyperalgesia. Activation or excitation of nociceptive nerve endings or fibers by these stimuli involves

the generation of action potentials, which then travel to the central nervous system and may induce pain sensation.³⁷ The formalin test, which implies injection of a formalin solution in the rat paw, indicates the excitation of nociceptors in previously sensitized animals by NTG administration. We assessed in our study both the first phase (the nociception phase) which consisted of direct action of a noxious stimulus modulated by a gate control mechanism and the second phase dominated by inflammation mechanisms.²⁷ On the other hand, experimental studies showed that activation of the spinal 5-HT1A receptor by its selective agonists induced an antinociceptive effect in the increasing temperature hot-plate test in rats and produced a dose-dependent decrease in N-methyl-D-aspartate receptor (glutamate specific receptors)-mediated behavior of rats.³⁸

The effect of ST

Because one of the most important mechanisms of migraine attack is a sudden decrease in 5-HT level, we tried to observe if there is a connection between the ST administration and the oxidative stress amplitude in an experimental migraine model. Our results showed that the ST administration significantly improved the oxidative stress parameters (see Tables 2 and 3). It was previously demonstrated that ST could repress the nociceptive pain by decreasing the activity of calcitonin gene-related protein via reducing NO formation.³⁹ The 5-HT1B/1D agonist, ST (0.1, 1.0 and 10 nmol), also inhibited paw edema induced by experimental inflammation in rats, proving its anti-inflammatory properties.⁴⁰ In our experimental study, ST was able to reduce the nociception

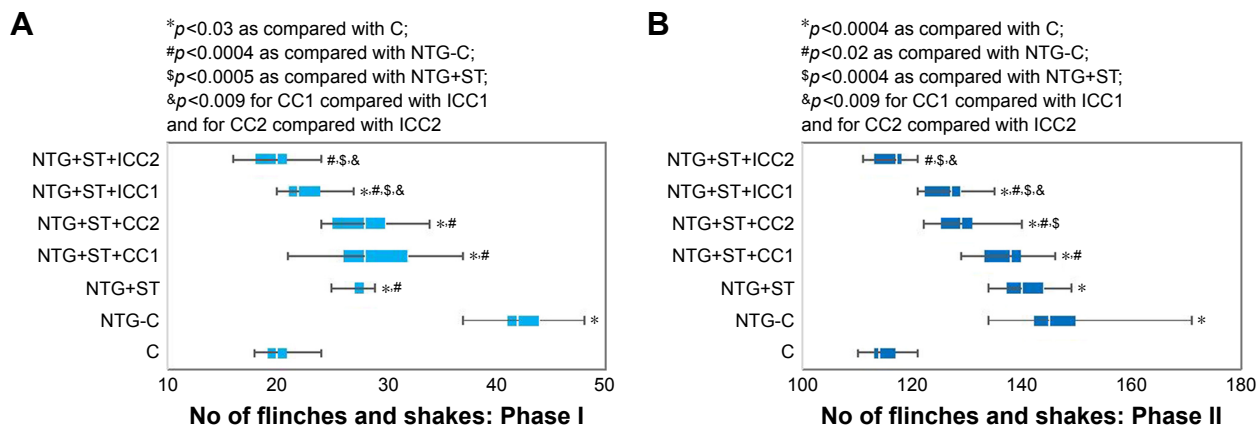


Figure 3 Number of flinches and shakes by groups: (A) Phase I, (B) Phase II. The box is given by the values of the first and third quartiles, the mid-line is given by the value of the median, and the whiskers are the minimum and maximum values. C is the control group (no medication), NTG-C is the group treated with nitroglycerin (NTG), NTG+ST is the group treated with NTG and sumatriptan (ST), NTG+ST+CC1 is the group treated with NTG+ST and curcumin 1 mg/100 g body weight (bw), NTG+ST+CC2 is the group treated with NTG+ST and curcumin 2 mg/100 g bw, NTG+ST+ICC1 is the group treated with NTG+ST and liposomal curcumin 1 mg/100 g bw, and NTG+ST+ICC2 is the group treated with NTG+ST and liposomal curcumin 2 mg/100 g bw.

Abbreviations: C, control; CC, curcumin; ICC, liposomal curcumin; NTG, nitroglycerin; Q1, 25th percentile; Q3, 75th percentile; ST, sumatriptan.

process in both nociceptive phases (even in the second one dominated by an inflammation mechanism), as shown, by significantly decreasing the number of flinches and shakes after the formalin test (see Table 4). Furthermore, the reduction in vasodilation associated with neurogenic inflammation can lead to reduction of the nociceptive process. ST mediates a vasopressor response in the systemic circulation through 5-HT₁ receptors, 5 min after administration.²¹ ST has vasoconstrictive properties, as well as effects on neurogenic inflammation by decreasing the release of important molecules involved in neurogenic inflammation (such as substance P and the calcitonin gene-related peptide).⁴¹ The rapid action of ST can be translated in a longer anti-nociceptive effect by combination with nonsteroidal anti-inflammatory drugs like naproxen due to a longer effect on the inflammatory process.⁴²

The effect of liposomal curcumin

Due to curcumin's anti-inflammatory⁴³ and antioxidative properties⁷ as well as its lack of toxicity and neuroprotective effect,⁴⁴ we investigated the combination of the rapid effect of ST on the nociception process with the effects of curcumin in parenteral administration.

Both pharmaceutical forms (solution and liposomal curcumin) were effective in treating experimental migraine and reduced the nociceptive process (as indicated by a significantly decreased number of flinches and shakes in the groups treated with CC solution [CC1 and CC2]). Furthermore, liposomal curcumin, at both concentrations, significantly reduced the nociceptive process compared to the control groups (C group, NTG-C group, and NTG+ST group), being more efficient than the CC solutions. In an experimental pain study, the simultaneous oral administration of curcumin with the intraperitoneal administration of tramadol significantly attenuated mechanical allodynia and thermal hyperalgesia.⁴⁵ Curcumin's anti-nociceptive mechanism is not clear but has proved to be part of the mechanisms involved in CX3CR1 expression, Mu and Delta opioid receptors, 5-HT (1A) receptors, or TNF- α expression.⁴⁶ Curcumin was also found to attenuate the capsaicin-induced transient receptor potential of vanilloid 1 in experimental pain hypersensitivity.⁴⁷ The intraperitoneal administration of curcumin blocked capsaicin-induced thermal hyperalgesia in a dose-dependent manner in trigeminal ganglion neurons.⁴⁷

Curcumin exhibits stronger analgesic effects when it is administered parenterally in a liposomal form. Several previous studies demonstrated that it is possible to enhance the biodistribution of curcumin by encapsulation in various

nanocarriers. The antinociceptive effect of curcumin nanoparticles in the experimental migraine model could also result from its ability to reduce neurogenic inflammation.^{48–50} Curcumin nanoformulation successfully inhibited experimental inflammation in a rat model, but at the same time, the employed concentration did not cause adverse effects on the brain tissue, as indicated by the brain lactic acid concentrations and measured neuronal apoptosis.⁵¹

The antioxidant effect of curcumin in addition to ST treatment was observed for all investigated groups (see Tables 2 and 3). The liposomal curcumin exerted a significantly greater antioxidant activity than the curcumin solution (see Tables 2 and 3). Its antioxidant effect could be attributed to its ability to scavenge free radicals such as hydroxyl radicals and peroxy radicals.^{52–54} Curcumin can also directly interact with the superoxide anion, as shown by electron paramagnetic resonance, and can inhibit the activity of nuclear factor-kappaB (NF-kappaB), which is involved in the transcription of proinflammatory mediators.⁵⁵ According to our results, the antioxidant effect of liposomal curcumin was significantly higher than that of the curcumin solution, for all the tested parameters (Figure 1). Data indicated that the MDA production, which reflects the degree of tissue and cell damage caused by lipid peroxidation, was reduced after the administration of curcumin, especially in the groups that received the liposomal formulation (Figure 1A). The decrease in MDA values after the administration of liposomal curcumin was shown by other researchers in oxidative stress conditions induced by experimental renal ischemia.⁵⁶ The ability to reduce the NO formation has also been demonstrated in previous studies.^{57,58} It has been shown that in brain tissue, the inhibition of inducible nitric oxide synthase can be closely correlated with increasing concentrations of the administered curcuminoids.⁵⁹ Curcumin can also reduce the amount of nitrite formed by the reaction between oxygen and nitric oxide,⁶⁰ which is an important mechanism of reducing oxidative stress. In addition, multifunctional nanocarriers seem to play a key role, considering that these pharmaceutical forms show enhanced characteristics such as biocompatibility and also have limited side effects.⁶¹ The administration of curcumin encapsulated in nanocarriers led to an elevated antioxidant effect compared to the free curcumin.^{62,63} The neuroprotective effect of curcumin nanoformulations after BBB disruption⁶⁴ can also be important since in migraine attacks there is an increased NO release associated with vasodilation⁶⁵ that can lead to BBB hyperpermeability.^{66,67} The neuroprotective effect of nanocurcumin combined with omega-3 fatty

acids, in an experimental migraine model, has also been reported by Abdolahi et al, and has been attributed to its anti-inflammatory properties that occur as a result of TNF- α serum level reduction.⁶⁸ The effect on NOx production was also more important for the liposomal curcumin group in our study (Figure 1B). The improvement of TOS (Figure 1C) and TAC (Figure 2B) parameters when curcumin was in addition to ST treatment had been demonstrated for all our study groups, with more significant results obtained for liposomal curcumin (ICC groups). The reduction of TOS and improvement of TAC in the curcumin-treated groups was also demonstrated in an experimental ovarian ischemia-reperfusion model in rats.⁶⁹ The effect of nanocurcumin on TOS and TAC parameters have not been reported in previous studies. Thiols that are able to mediate redox signaling processes as response to oxidative stress were significantly increased in our study groups, especially for the groups that received liposomal curcumin (Figure 2A). The underlying mechanism for the increase in the thiol level after curcumin administration could be the induction of glutathione biosynthesis and inhibition of the NF-kappaB activation.^{70,71} Multifunctional glutathione, particularly in reduced form (GSH), is an intracellular thiol found in all tissues with an important role in protecting against oxidative stress.⁷² Our study demonstrated that the administration of liposomal curcumin (at both concentrations) was able to significantly enhance the thiol level compared to the control groups, while ST alone or ST associated with the curcumin solution did not significantly improve the thiol level. This phenomenon can be explained by an increased metabolic stability of liposomal curcumin and a better tissue distribution, which is associated with a lower rate of metabolization to tetrahydrocurcumin and enhanced antioxidative properties.⁷³

Study limitations

A rigorous experimental design was applied to assess the effect of the intravenous administration of curcumin-loaded liposomes when in addition to ST treatment in an experimental animal migraine model. Despite the rigor of the applied experimental design, several limitations can be listed. First, the formalin test for nociception may not be the ideal test since the results could be affected by peripheral tissue temperature or other factors influencing the peripheral inflammation.²⁷ A computational model developed by Fischer et al⁷⁴ supports the biphasic response, the interphase being the result of hyperpolarization and transient inactivation, followed by recovery prior to decrease in formalin concentration below threshold. However, the 1% formalin injection used in our

design assured the absence of long-lasting hypersensitivity and peripheral inflammation (reported for 2% and 5% in a spinal nerve injury model)⁷⁵ and thus the comfort of animals. Despite its limitations, the formalin test is still currently used in the evaluation of both pain and inflammation in animal models.^{76,77} Second, the concentration of curcumin and/or ST in the blood, as well as in the brain tissue, was not quantified. This kind of measurements could bring useful information, but a separate design using different forms of curcumin must be tested. Our research group, therefore, sets to pursue this goal in further studies.

Conclusion

The liposomal curcumin at a dose of 2 mg/100 g bw seems to enhance the antioxidative effect of ST. However, further studies, including on liposomal curcumin alone, are needed to confirm our findings. The use of nanotechnology in medicine could be an appropriate means to enhance the efficiency of different treatments. The difference between ST in single administration and in combination with conventional curcumin is marginal, but the association with liposomal curcumin proved to be efficient in our experimental migraine model. The efficiency of liposomal curcumin has to be tested in clinical conditions and could represent an improved therapeutic modality for migraine attacks. Therefore, liposomal curcumin could be useful for improving the bioavailability and efficacy of this compound.

Disclosure

The authors report no conflicts of interest in this work.

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