Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Assessment Of Spanlastic Vesicles Of Zolmitriptan For Treating Migraine In Rats

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy

Nagla Ahmed El-Nabarawy[1]
Mahmoud Hassan Teaima[2]
Doaa Ahmed Helal[3]

[1]National Egyptian Center of Environmental & Toxicological Research (NECTR), Faculty of Medicine, Cairo University, Cairo, Egypt; [2]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt; [3]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Fayoum University, Elfayoum, Egypt

Objective: To develop and evaluate zolmitriptan spanlastics (Zol SLs) as a brain-targeted antimigraine delivery system. Spanlastics (SLs) prepared using span 60: tween 80 (70:30%, respectively) gave the highest percentage of entrapment efficiency (EE%).

Materials and methods: A total of 60 adult male Wistar albino rats were divided into six groups (n=10 rats/group). Group 1 (Control) comprised rats serving as a negative control. Group 2 was treated with glyceryl trinitrate (NTG) and served as the positive control. Groups 3 (NTG+Zol com), Group 4 (NTG+Zol sol) and Group 5 (NTG+Zol SLs) received commercial zolmitriptan orally, zolmitriptan solution intranasally and Zol SLs F5 intranasally, respectively, 30 min before NTG. Group 6 (Zol SLs) comprised normal rats that received only Zol SLs intranasally.

Results: We found decreased T_{max}, increased C_{max}, AUC_{0-6}, $AUC_{0-\infty}$ and ameliorated behaviour in rats (head scratching) treated with intranasal SLs compared to oral commercial zolmitriptan.

Conclusion: Our study substantiates the enhanced efficacy of Zol SLs in brain targeting for migraine treatment.

Keywords: zolmitriptan, Zol, intranasal delivery, brain targeting, spanlastics, SLs, pharmacokinetics, pharmacodynamics, migraine

Introduction

Migraine is a chronic relapsing and remitting brain disorder characterized by recurrent debilitating headache attacks that occur for at least 15 days/month. Apart from psychosis, dementia and quadriplegia, migraine is one of the highly disabling neurologic conditions.[1–3] The high prevalence of migraine and the noticeably impaired quality of the sufferer's life impose a heavy societal burden in terms of high medical costs, work productivity loss and unemployment.[4]

Pathogenesis of migraine involves an imbalance in activity between brain stem nuclei controlling the trigeminovascular nociceptive system and vascular control.[5] Trigeminovascular system activation leads to the release of a number of vasoactive neuropeptides, mainly calcitonin gene-related peptide (CGRP) and substance P*,[6,7] which further dilate cerebral blood vessels and elicit an inflammatory response causing pain.[8] CGRP is firmly established as a key player in migraine[9] and also stimulates the production and release of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) from human lymphocytes.[10]

Nuclear factor-κB (NF-κB) is a redox-sensitive transcription factor that regulates the expression of a range of inflammatory genes and has been studied as a molecular target for migraine therapy.[11] Nitric oxide (NO) also plays a vital role in

Correspondence: Nagla Ahmed El-Nabarawy
National Egyptian Center of Environmental & Toxicological Research (NECTR), Faculty of Medicine, Cairo University, Kasr A Ainy Street, Cairo 11562, Egypt
Email n.a.nabarawy@gmail.com

submit your manuscript | www.dovepress.com
DovePress
http://doi.org/10.2147/DDDT.S220473

migraine,[12] and both inducible NOSand neuronal NOS have been essentially involved in the pathogenesis of migraine.[13]

Migraine patients striving for relief mostly regard the rapid onset of drug action a top priority. Therefore, pharmacokinetic parameters related to fast onset of action are of significant concern while developing antimigraine formulations.[14,15]

Zolmitriptan is a second-generation triptan derivative that is used principally for acute migraine and cluster headache treatment. It exerts a selective agonistic effect on 5HT1B/1D serotonin receptors, centrally and peripherally, thereby hampering the activation of the trigeminovascular system, reducing neurogenic inflammation, constricting dilated cerebral blood vessels and curbing vasoactive neuropeptide release. Zolmitriptan possesses an oral and nasal bioavailability of almost 40% and a plasma half-life of 3 hrs.[14–16]

Based on the importance of the central action of zolmitriptan, there is special concern for enhancing its availability and transport rate to the brain since one of the greatest challenges of targeting drugs to the CNS is the tight blood–brain barrier (BBB). The intranasal route is one of the different pathways for delivering the drug into the brain: the drug crosses the BBB, through the olfactory region and the trigeminal pathway where it is transported directly from nasal cavity to the central nervous system.[17]

Intranasal route provides an excellent site for rapid absorption of centrally acting drugs. In addition, nasal application circumvents first-pass elimination, degradation and irritation in GIT, and may be employed routinely without any pain. As it is noninvasive, it reduces the risk of infection compared to IV administration.[18]

Nanoparticles have lately been considered as suitable carriers for maximizing the brain targeting potential of many CNS active drugs[19] as spanlastics (SLs) which are formed of a Span® and an edge activator (EA). SLs can be used to deliver both hydrophilic and hydrophobic drugs which are encapsulated in interior hydrophilic compartment and outer lipid layer, respectively.[18] Therefore, zolmitriptan can be entrapped in SL where the transport across the BBB to reach the brain is based on the characteristics of SL dispersion and not on that of the therapeutic agent; also, they have the ability to squeeze themselves between membranes.

The objective of the present study was to develop and evaluate the efficacy of intranasally delivered Zol SLs, compared to the orally delivered drug, in a rat model of glyceryl trinitrate (NTG)-induced migraine.

Materials And Methods

Materials

Zolmitriptan was purchased from BMR Pharma and Chemical Company (India). Sorbitan monoesters (Span 60), polysorbates (Tween 60 and 80), ethanol and methanol were kindly supplied by the Egyptian International Pharmaceutical Industries Co. (EPICO, Egypt). Commercial zolmitriptan (Zomig®, 2.5 mg tablets) was obtained from Vichy Laboratories (France). NTG as Nitronal® aqueous solution (1 mg/mL) was obtained from POHL-BOSKAMP (Hohenlockstedt, Germany). Water for liquid chromatography, HPLC and spectrophotometry was manufactured by Honeywell Burdick & Jackson (Mexico City, Mexico). Dichloromethane (CHROMASOL®, for HPLC containing amylene as stabilizer) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Methods

Preparation Of Zol SLs

Zol SLs systems composed of Span 60 and EAs (namely Tween 60 and Tween 80) were prepared using different weight ratios of Span 60: EA (80:20, 70:30 and 50:50 w:w) (Table 1), using ethanol injection method as described by Kakkar and Kaur (2011).[20] Briefly, Span 60 and calculated amount of zolmitriptan were dissolved in 4 ml ethanol and then the dissolved solution was injected dropwise at a constant rate into a preheated aqueous phase (66°C) containing EA. It was continuously stirred using a magnetic stirrer (Model MSH-20D; Witeg Labortechnik GmbH Germany) at 1000 rpm for 45 min, and Zol SLs dispersions were obtained.

In Vitro Evaluation Of The Prepared SLs

Calculation Of Percentage Of Entrapment Efficiency (EE%)

The SL vesicles were disrupted by methanol and release the drug. Total drug content of the prepared formulae was determined by dissolving 1 mL of SL dispersion in 10 mL methanol and then zolmitriptan was quantified spectrophotometrically using UV/VIS spectrophotometer (model UV-1601PC, Shimadzu, Kyoto, Japan) at λ_{max} 283 nm.[21] Entrapment efficiency was determined as follows:[18,22,23]

$$\text{Entrapment efficiency of zolmitriptan} = (\text{Amount trapped}/\text{Total amount of zolmitriptan}) \times 100 \quad (1)$$

Table 1 Composition, Mean Particle Size, Polydispersity Index (PDI), Zeta (ζ) Potential And Entrapment Efficiency Of Zolmitriptan Spanlastics Dispersions

Formula	Composition	SAA: EA Weight Ratio	Particle Size (nm)	PDI	Zeta (ζ) Potential (mV)	E.E(%)
F1	S60: T60	80: 20	305.02±190	0.35±0.13	−35.35±2.37	63.05±4.50
F2	S60: T60	70: 30	160.5±90.19	0.24±0.09	−31.9±4.81	77.7±2.64
F3	S60: T60	50: 50	143.06±95.09	0.46±0.11	−38.81±2.91	59.20±3.51
F4	S60: T80	80: 20	238.26±118.06	0.61±0.24	−38.76±3.3	82.75±4.96
F5	S60: T80	70: 30	268.98±189.07	0.38±0.15	−32.92±3.77	89.58±6.03
F6	S60: T80	50: 50	235.03±75.06	0.59±0.19	−31.2±4.18	51.00±5.03

Note: Results are the mean±SD.

Particle size (PS) analysis, zeta (ζ)-potential measurements, polydispersity index (PDI) were calculated by conducting morphological examination.

The vesicular size, ζ-potential, and PDI of diluted Zol SL dispersion were determined using dynamic light scattering with Malvern Zetasizer (Malvern Instrument Ltd., UK).[24] The PDI was used to show the homogeneity of PS.[25]

A negatively stained Zol SL with 2% (w/v) phosphotungstic acid solution was visualized using a TEM (Jeol – JXA-840A – Electron Microscope – Japan).[26]

In Vitro Dissolution Of Zol SLs

The dissolution profile of zolmitriptan powder, Zol SLs and the commercial Zomig® tablets were determined using the membrane diffusion technique. An accurately weighed amount of the prepared Zol SL dispersion system equivalent to 2.5 mg zolmitriptan was transferred to a glass cylinder having the length 8 cm and diameter 2.5 cm with surface area 4.91 cm^2 and fitted at its lower end with presoaked dialysis membrane on which the SL spread over (Spectra/Pore dialysis membrane, 12,000–14,000 Mwt cut-off (Spectrum Laboratories Inc., USA). The glass cylinder was attached to the shaft of the dissolution apparatus and then was placed in vessels of the U.S.P. Dissolution Tester. Dissolution was carried out in triplicate using 500 mL phosphate buffer at pH 6.8, the basket was rotated at 100 rpm, and the dissolution medium temperature was maintained at 37°C. Aliquots of 5 mL were withdrawn at 5, 10, 15, 30, 45, 60, 90, 120, 150, 180 and 240 min, and the media were replaced. Samples were filtered using 0.45 Millipore filters before measuring their absorbance at λ_{max} 283 nm.

In Vivo Evaluation Of Zol SLs

A total of 60 adult male Wistar albino rats (8 weeks old, weight: 200–250 g), obtained from the breeding unit of Faculty of Pharmacy, Cairo University, were used in the in vivo study. NTG was used to induce migraine in rats at a dose of 10 mg/kg through intraperitoneal injection.[27–30] Successful model establishment was confirmed by the presence of ear flushing, scratching the head frequently using forelimbs, increased activity to climb the cage, biting tails and reciprocating motion.[30] Zolmitriptan was administered to treatment groups at a dose of 5 mg/kg, 30 min before the intraperitoneal injection of NTG. Six hours after NTG administration, rats were sacrificed.[27,28]

Ethics Approval

All the experimental procedures used in the present study were conducted according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and were approved by the research ethics committee for experimental and clinical studies at Faculty of Pharmacy, Cairo University (Approval date: 28/03/2016, Permit number: PI 1645).

Study Design

Rats were randomly allocated into six groups (n=10 rats/group). Group 1 (Control) comprised normal rats serving as a negative control. Group 2 (NTG) was treated with NTG and served as the positive control. Groups 3 (NTG +Zol com), 4 (NTG+Zol sol) and 5 (NTG+Zol SLs) received commercial zolmitriptan orally, zolmitriptan solution intranasally and Zol SLs F5 intranasally, respectively, 30 min before NTG. Group 6 (Zol SLs) comprised normal rats that received only Zol SLs intranasally.

Pharmacokinetic Study

The pharmacokinetic study was performed on 60 rats (10 rats/group).

Zolmitriptan Analysis In Plasma

Quantitative evaluation was done by analysis of plasma zolmitriptan concentration for NTG+Zol com, NTG+Zol sol and NTG+Zol SLs groups. Blood samples of 0.2 mL each were collected into heparinized microcentrifuge

tubes, through the retro-orbital vein from alternative eye at each time interval. Time intervals of blood sampling were 0.5, 1, 1.5, 2, 2.5, 3, 4, 5 and 6 hrs after zolmitriptan administration. The blood samples were centrifuged at 4000 rpm for 20 min to separate the plasma that was stored at −20°C until drug analysis. Frozen plasma samples were thawed at room temperature.

Zolmitriptan Calibration Standards Extraction

One milliliter plasma was added to 4 mL methyl tertiary butyl ether, then vortexed for 1 min, centrifuged at 4000 rpm for 10 min, and the supernatant was evaporated at 45°C. A volume of 250 µL caffeine internal standard (10 µg/mL) was added to the residue. Finally, injection into the HPLC chromatograph was performed (HPLC Device: Shimadzu, Autosampler: SiL 20 A, System controller: SCL-10 A vp, Detector: SPD-10 A vp UV-Vis, Pump: LC-10 AD vp liquid chromatograph, Degasser: DGU-12 A, Column: Scharlau 250x4.6 mm P/N 066-B6Y803).

Chromatographic Conditions

Mobile phase: buffer:acetonitrile:MeOH (55:20:25), wavelength: 225 nm, flow rate (mL/min): 1, column: C18 300×46, internal standard: caffeine.

Pharmacokinetic Parameter Calculations

Pharmacokinetic analysis of plasma zolmitriptan concentration was performed using pharmacokinetic add-in package for Microsoft excel 2016[31] applying non-compartmental analysis. The determined pharmacokinetic parameters include maximum concentration in plasma (C_{max}), the time for maximum concentration in plasma (T_{max}), the area under plasma concentration versus time curve from 0 to 6 hrs (AUC_{0-6}), area under the plasma concentration versus time curve from zero to infinity ($AUC_{0-\infty}$), the area under momental plasma concentration versus time curve from 0 to 6 hrs ($AUMC_{0-6}$), area under momental plasma concentration versus time curve from zero to infinity ($AUMC_{0-\infty}$), mean residence time (MRT) and half-life ($t_{1/2}$).

Behavioral Study

The behavioral changes in the treated rats were monitored through counting the number of head scratchings during the 1st, 2nd, 3rd and 4th hrs after NTG administration.

Statistical Analysis

Data are presented as mean±SD and were analysed using one-way ANOVA with extended least significant difference post hoc tests (subsequent multiple comparisons using Tukey's test), except for T_{max} data which were analysed by non-parametric Kruskal–Wallis test. All statistical tests were performed using IBM SPSS Statistics version 23, 64-bit edition, NY, USA. A *P* value of less than 0.05 was considered statistically significant.

Results And Discussion

In Vitro Evaluation Of The Prepared Zol SLs

Table 1 shows the EE%, mean PS (nm), size distribution (PDI), and zeta (ζ)-potential distribution (ZP) of different vesicles. Zolmitriptan was successfully entrapped in all SL vesicles prepared with different ratios of EA with Span 60. The EE% varied between 51.00±5.03% and 89.58±6.03%.

SL vesicles (F5) prepared from span 60 and Tween 80 (70:30 respectively) gave the highest EE% of 89.58 ±6.03% that was statistically significant compared to the other formulae. The EE% showed the following trend: F5 > F4 > F2 > F1> F3> F6.

The PS of formed vesicles ranged from 143.06±95.09 nm to 305.02±190 nm. The homogeneity of Zol SL dispersion was evaluated by the PDI parameter which ranged from 0.24 ±0.09 to 0.61±0.24; values less than 0.4 indicated a homogeneous population, while values more than 0.4 indicated a broad distribution[32]. ZP values ranged from −31.2±4.18 to −32.92±3.77 mV which give stable SL vesicles due to the presence of electrical repulsion; the higher the value of ζ-potential, the more stable the SL vesicles.[18,25,32]

The previous results reveal that the SL vesicles prepared using S60:T80 (70:30 respectively) (F5) are superior to other prepared formulae.

The prepared SLs (F5) were spherical in shape using the transmission electron microscopy (TEM) images (Figure 1).

In Vitro Dissolution Of Zol SLs

The dissolution profiles of zolmitriptan powder, Zol SLs and commercial zolmitriptan tablets in phosphate buffer (pH 6.8) are shown in Figure 2. The cumulative % of zolmitriptan dissolved after 1 hr (Q_{1hr}) and 6 hrs (Q_{6hr}) for different formulae can be ranked as follows: zolmitriptan powder > F5 Zol SL dispersion > commercial zolmitriptan tablets.

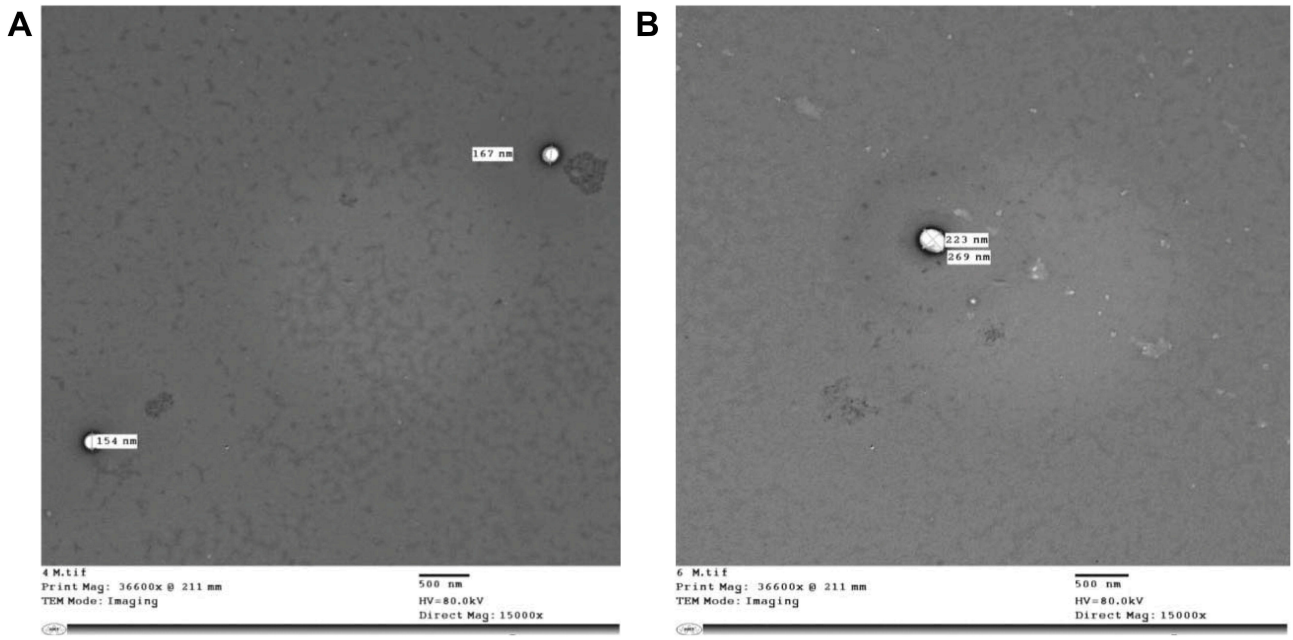

Figure 1 (**A**) TEM image of zolmitriptan spanlastics prepared using F2. (**B**) TEM image of zolmitriptan spanlastics prepared using F5.

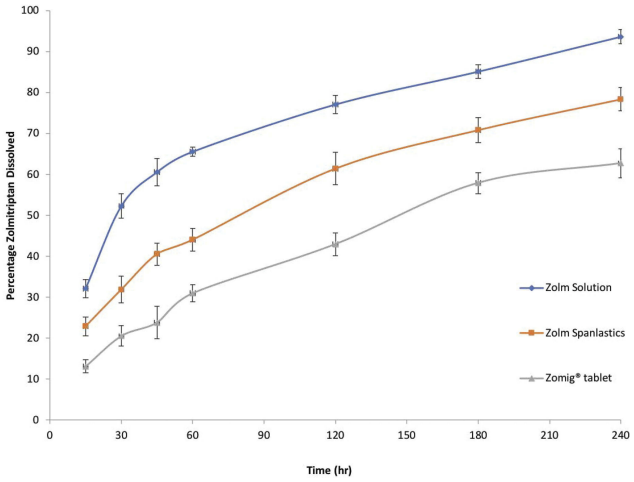

Figure 2 Mean percentage of zolmitriptan dissolved from zolmitriptan powder, zolmitriptan spanlastic dispersion F5 and commercial zolmitriptan (2.5 mg tablets).

The dissolution results indicated lower zolmitriptan release from an SL formula when compared with free zolmitriptan powder, which could be attributed to the reduced leakage and permeability of SL formulations. On the contrary, the release of zolmitriptan from F5 SL vesicles was faster than that from commercial zolmitriptan.

In all formulae, it was found that Q_{1hr} and Q_{6hrs} from SLs have non-significantly lower values ($p > 0.05$) compared to the pure zolmitriptan powder and non-significantly higher values compared to the commercial zolmitriptan tablets ($p > 0.05$).

In Vivo Evaluation Of Zol SLs

Pharmacokinetic Study

Quantitative Evaluation Of Zolmitriptan In Rat Plasma By HPLC

A sample of the HPLC chromatograms of zolmitriptan in plasma is presented in Figure 3. The mean zolmitriptan plasma levels in rats after administration of the different zolmitriptan treatments versus time are presented in Figure 4. The pharmacokinetic parameters of different zolmitriptan treatments, such as C_{max}, T_{max}, AUC_{0-6}, $AUC_{0-\infty}$, $AUMC_{0-6}$, $AUMC_{0-\infty}$, MRT and $T_{1/2}$, are presented in Table 3.

After administration of oral commercial zolmitriptan, intranasal zolmitriptan solution and intranasal F5 Zol SL dispersion, the maximum plasma concentrations were observed at 0.5 hr, 1 hr, and 1.5 hrs, respectively. The mean maximum plasma drug concentration in the NTG+Zol SLs group was higher than those of NTG+Zol com and NTG+Zol sol groups. The mean time to reach the peak concentration (T_{max}) was significantly lower in the NTG+Zol sol and NTG +Zol SLs groups compared to the NTG+Zol com group. These findings, along with the AUC_{0-6}, $AUC_{0-\infty}$, $AUMC_{0-6}$ and $AUMC_{0-\infty}$ results, suggest that the oral commercial zolmitriptan product showed the lowest rate and extent of drug absorption, whereas intranasal Zol SL dispersion showed the highest rate and extent of drug absorption.

The differences between the values of log C_{max}, log AUC_{0-6}, log $AUC_{0-\infty}$, log $AUMC_{0-6}$, log $AUMC_{0-\infty}$ and

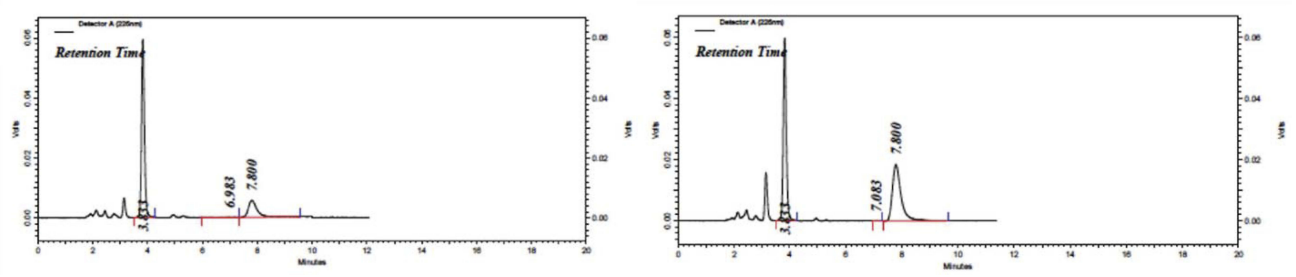

Figure 3 Representative HPLC chromatograms of zolmitriptan in rat plasma.

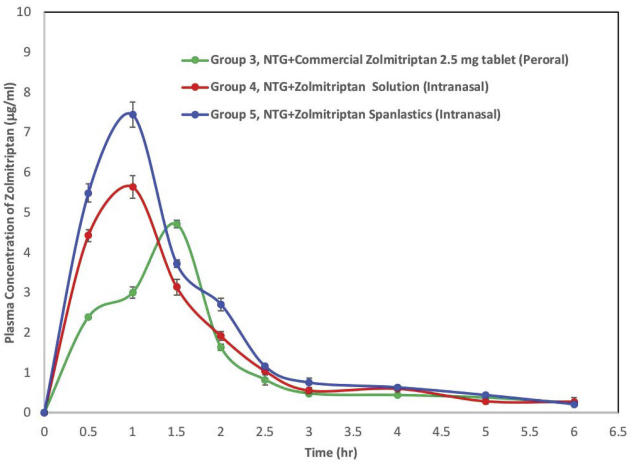

Figure 4 Mean plasma level of zolmitriptan following administration of intranasal zolmitriptan.

T_{max} calculated for NTG+Zol com, NTG+Zol sol and NTG+Zol SLs groups were statistically significant as shown in Table 3.

Behavioral Study

As shown in Table 2, the number of head scratchings in the NTG group was significantly greater compared to the negative control group. The number of head scratchings in NTG+Zol com, NTG+Zol sol and NTG+Zol SLs groups decreased significantly compared to the NTG group, with the NTG+Zol SLs group showing a significantly lower number compared to the NTG+Zol com and NTG+Zol sol groups.

Furthermore, our results revealed variation in head scratching frequency over different time intervals. During the first hour, the negative control group showed 10.83 ±2.48, whereas scratching reached a maximal level in the NTG group 67.50±14.89. During the second hour, the head scratching frequency was sustained at the peak level in the NTG group 52.50±11.72. In addition, a significant difference in head scratching frequency was observed between zolmitriptan-treated groups (NTG+Zol Com, NTG+Zol sol, NTG+Zol SLs) which were 37.33 ±0.81, 24.83±1.16 and 13.67±0.81 respectively compared to NTG group 52.50±11.72. On the other hand during the third hour, the head scratching frequency decreased in the NTG group to 32.83 ± 7.05 but was still significantly different from the control group 9.83±2.31 and zolmitriptan treated groups (NTG+Zol Com, NTG+Zol sol, NTG +Zol SLs) which were 29.17±1.16, 21.50±0.54 and 11.83 ±0.98 respectively. Also, during the fourth hour, the NTG group showed gradual decrease in head scratching frequency to 25.17±6.36. However, the number of scratching remains at its peak compared with the negative control group.

The behaviours of rats can reflect the influence of treatment. NTG (NTG also referred to as nitro-glycerine) is widely used as an exogenous NO donor which depends on the mitochondrion. Mitochondrial aldehyde dehydrogenase-2facilitates the formation of NTG and generates 1,2-glyceryl dinitrate and nitrite then metabolizes to NO. Two main pathways have been shown for NTG formation: one based on NTG producing NO which directly causes vasodilatation, and the other is based on detoxification that produces inorganic nitrite anions.[33]

Also, our results are compatible with those reported by Greco et al[34] who found that NTG is a potent vasodilator and NO donor. In addition, NTG causes upregulation of pro-inflammatory mediators, macrophage activation, edema formation and mast cell degranulation by a direct action on meningeal tissues[35] Moreover, Jim[36] suggested that NF-kB is a mediator of pro-inflammatory cytokines and other neurochemical signals leading to migraine attacks in the presence of NTG induced increase NF-kB transcriptional activity. In addition, our results corroborate with Monteith and Goadsby[37] who found that zolmitriptan was developed with the strategy to create a more lipophilic compound with faster absorption and better ability to cross the BBB than other triptans. It also acts through stimulating vascular 5-HT_{1B} receptors, thus

Table 2 Number Of Head Scratching In Different Groups

Group	n	Number Of Head Scratching			
		0–1 hr	1–2 hrs	2–3 hrs	3–4 hrs
1, Negative Control (No NTG+No Medication)	15	10.83±2.48	10.83±2.48	9.83±2.31	7.83±1.72
2, Positive Control (NTG+No Medication)	15	67.50±14.89$^{\#}$	52.50±11.72$^{\#}$	32.83±7.05$^{\#}$	25.17±6.36$^{\#}$
3, NTG+Commercial Zomig® 2.5 mg tablet (per oral)	15	46.83±1.47$^{*\#}$	37.33±0.81$^{*\#}$	29.17±1.16$^{*\#}$	25±0.89$^{*\#}$
4, NTG+Zolmitriptan solution (intranasal)	15	33±1.26$^{*\#\$}$	24.83±1.16$^{*\#\$}$	21.50±0.54$^{*\#\$}$	16.83±0.75$^{*\#\$}$
5, NTG+ZolmitriptanSpanlastic dispersion (intranasal)	15	19±2.19$^{*\#\$}$†	13.67±0.81$^{*\#\$}$†	11.83±0.98$^{*\#\$}$†	8.83±0.75$^{*\#\$}$†
6, (No NTG+ZolmitriptanSpanlasticdispersion (intranasal)	15	11.27±3.17*	10.62±2.59*	10.91±1.15*	8.22±2.25*

Notes: $\bar{X}$± SD, n = 15, $^{\#}$ p < 0.05 vs negative control group 1, * P < 0.05 vs positive control group 2, $^{\$}$ p < 0.05 vs commercial group 3, † p < 0.05 vs zolmitriptan solution group 4.

Table 3 Pharmacokinetic Parameters Of Zolmitriptan Following Administration Of Peroralzolmitriptan Commercial Zomig® 2.5 Mg Tablet (Group 3), Intranasal Zolmitriptan Solution (Group 4) And Intranasal zolmitriptanSpanlastic Dispersion (Group 5) Versus Time

Pharmacokinetic Parameter	Group 3, NTG+Commercial Zomig® 2.5 mg Tablet (Peroral)	Group 4, NTG +Zolmitriptan Solution (Intranasal)	Group 5, NTG+Zolmitriptan Spanlastic Dispersion (Intranasal)
T_{max} (hr)	1.50	1.00	1.00
C_{max} (μg mL^{-1})	4.71±0.1	5.64±0.31	7.45±0.31
AUC_{0-6} (μg hr mL^{-1})	7.59±0.14	9.51±0.27	12.08±0.34
$AUC_{0-\infty}$ (μg hr mL^{-1})	8.20±0.15	10.20±0.55	12.48±0.33
$AUMC_{0-6}$ (μg hr^2 mL^{-1})	13.77±0.47	15.36±0.54	19.26±0.49
$AUMC_{0-\infty}$ (μg hr^2 mL^{-1})	17.48±0.87	19.47±2.22	21.62±0.73
$T_{1/2}$ (hr)	1.76±0.45	1.76±0.50	1.28±0.10
MRT (hr)	2.13±0.08	1.90±0.12	1.73±0.05
Vd (L)	2.90±0.96	6.95±5.40	3.54±1.70
Cl (mL/min)	18.69±4.52	39.51±26.01	31.24±14.35
K (hr-1)	0.40±0.04	0.43±0.15	0.55±0.05

Note: $\bar{X}$± SD, n = 6.

causing vasoconstriction of cerebral, meningeal, dural or pial vessels, and inhibition of dural neurogenic inflammation through stimulation of 5-HT_{1D} and 5-HT_{1F} receptors. In addition, stimulation of 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptors causes inhibition of trigeminal neurons in the brain stem and upper spinal cord.[38] Moreover, our results coincided with Akbari et al[39] who found that recent advances in non-ionic surfactant vesicles of zolmitriptan can be considered economically, chemically, physically stable and have potential advantages of phospholipid vesicles of being able to accommodate both water and lipid-soluble drug molecules, control their release, and serve as devices of numerous applications.

Nanoparticles and drug delivery increase the stability of the pharmaceutical agents and can be easily and inexpensively fabricated in large quantities. Nanoparticles have been investigated in biomedical and biotechnological areas and in drug delivery system for drug targeting. The advantages of targeted drug delivery to the specific site of the body paved the way for applying nanoparticles to achieve the type of drug delivery. The nasal route was adopted to exploit its avoidance of hepatic first-pass metabolism to increase the bioavailability. Therefore, the formulation of nose to brain Zol SL was selected to enhance permeability across BBB against nitroglycerine-induced migraine.[40]

The model of migraine induced by NTG has provided a growing body of information about pathophysiology of migraine headache and therapeutic targets.[28,41]

NTG triggers headache in normal subjects and induces migraine in patients.[42] In rodents, NTG-evoked hyperalgesia has been developed as a model for sensory hypersensitivity associated with migraine.[43,44]

Only a limited number of animal studies have investigated aspects of headache disorders which remains unexplored of pathogenesis. So, the selection for NTG model systems has a great field for research of migraine headache.

Anti-migraine medications such as triptans were tested on this model and showed moderate efficiency. But SL vesicles of zolmitriptan will yield exciting advances in the treatment of migraine attacks in the future. It was developed with the strategy to create a more lipophilic compound with faster absorption and better ability to cross BBB.[40] There is strong evidence to support the use of spanlastic vesicles of zolmitriptan who have had poor response to previous therapy. It is well effective across a wide range of migraine subtypes and well tolerated.

Finally, it was found that the intra-nasal administration of zolmitriptan as an SL dispersion enhanced the bioavailability of the drug by decreasing the time required to reach peak plasma concentration, increased the extent of absorption as well and its ability to cross the BBB and hence produced a superior ameliorative anti-migraine effect, behavior and pharmacokinetic profiles. The development of this new drug delivery system through the nasal route provides a faster and more efficient alternative to the commercially available oral tablet.

Conclusion

The present study substantiated that SL vesicles prepared using ethanol injection technique exhibited high zolmitriptan entrapment efficiency, a globule size in the nanometric range and faster in vitro dissolution compared to the commercial tablet. The in vivo findings demonstrated superior alleviation of migraine-related behavior by the SL formulation compared to the commercial product. In conclusion, intranasally delivered Zol SL vesicles represent a superior anti-migraine approach compared to the conventional tablet formulation.

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Edvinsson L, Villalón CM, Maassenvandenbrink A. Basic mechanisms of migraine and its acute treatment. *Pharmacol Ther*. 2012;136:319–333. doi:10.1016/j.pharmthera.2012.08.011
2. Goadsby PJ. Recent advances in understanding migraine mechanisms, molecules and therapeutics. *Trends Mol Med*. 2007;13:39–44. doi:10.1016/j.molmed.2006.11.005
3. May A, Schulte LH. Chronic migraine: risk factors, mechanisms and treatment. *Nat Rev Neurol*. 2016;12:455–464. doi:10.1038/nrneurol.2016.93
4. Marmura MJ, Silberstein SD, Schwedt TJ. The acute treatment of migraine in adults: the American Headache Society evidence assessment of migraine pharmacotherapies. *Headache*. 2015;55:3–20. doi:10.1111/head.12499
5. Weiller C, May A, Limmroth V, et al. Brain stem activation in spontaneous human migraine attacks. *Nat Med*. 1995;1:658–660. doi:10.1038/nm0795-658
6. Aggarwal M, Puri V, Puri S. Serotonin and CGRP in migraine. *Ann Neurosci*. 2012;19:88–94. doi:10.5214/ans.0972.7531.12190210
7. Kim G-M, Jin K-S, Chung C-S. Differential effects of corticosteroids on the expression of cyclooxygenase-2, tumour necrosis factor-alpha and matrix metalloproteinase-9 in an animal model of migraine. *Cephalalgia*. 2008;28:1179–1187. doi:10.1111/j.1468-2982.2008.01667.x
8. Moskowitz MA, Macfarlane R. Neurovascular and molecular mechanisms in migraine headaches. *Cerebrovasc Brain Metab Rev*. 1993;5:159–177.
9. Russo AF. Calcitonin Gene-Related Peptide (CGRP): a new target for migraine. *Annu Rev Pharmacol Toxicol*. 2015;55:533–552. doi:10.1146/annurev-pharmtox-010814-124701
10. Cuesta MC, Quintero L, Pons H, Substance S-RH. P and calcitonin gene-related peptide increase IL-1 beta, IL-6 and TNF alpha secretion from human peripheral blood mononuclear cells. *Neurochem Int*. 2002;40:301–306. doi:10.1016/S0197-0186(01)00094-8
11. Reuter U, Chiarugi A, Bolay H, Moskowitz M. Nuclear factor-kappaB as a molecular target for migraine therapy. *Headache J Head Face Pain*. 2003;43:426–427. doi:10.1046/j.1526-4610.2003.03085_11.x
12. Trainor DC, Jones RC. Headaches in explosive magazine workers. *Arch Environ Health*. 1966;12:231–234. doi:10.1080/00039896.1966.10664362
13. Pietrobon D, Striessnig J. Neurobiology of migraine. *Nat Rev Neurosci*. 2003;4:386–398. doi:10.1038/nrn1102
14. Shelke S, Shahi S, Jalalpure S, Dhamecha D. Poloxamer 407-based intranasal thermoreversible gel of zolmitriptan-loaded nanoethosomes: formulation, optimization, evaluation and permeation studies. *J Liposome Res*. 2016;26:313–323. doi:10.3109/08982104.2015.1132232
15. Girotra P, Singh SK, Kumar G. Development of zolmitriptan loaded PLGA/poloxamer nanoparticles for migraine using quality by design approach. *Int J Biol Macromol*. 2016;85:92–101. doi:10.1016/j.ijbiomac.2015.12.069
16. Lewis DW, Winner P, Hershey AD, Wasiewski WW. Efficacy of zolmitriptan nasal spray in adolescent migraine. *Pediatrics*. 2007;120:390–396. doi:10.1542/peds.2007-0085
17. El Nabarawi M, Abdelmonem R, Attia AM. Formulation and evaluation of intranasal granisetron hydrochloride spanlastic dispersions for postoperative and cancer associated therapies. *Inventi Impact Pharm Tech*. 2016;2016:126–131.
18. Abdelmonem R, el Nabarawi M, Attia A. Development of novel bioadhesive granisetron hydrochloride spanlastic gel and insert for brain targeting and study their effects on rats. *Drug Deliv*. 2018;25(1):70–77. doi:10.1080/10717544.2017.1413447
19. Jafarieh O, Md S, Ali M, et al. Design, characterization, and evaluation of intranasal delivery of ropinirole-loaded mucoadhesive nanoparticles for brain targeting. *Drug Dev Ind Pharm*. 2015;41:1674–1681. doi:10.3109/03639045.2014.991400
20. Kakkar S, Kaur IP. Spanlastics A novel nanovesicular carrier system for ocular delivery. *Int J Pharm*. 2011;413:202–210. doi:10.1016/j.ijpharm.2011.04.027
21. Acharjya SK, Rao MEB, Kumar BVVR, Annapurna MMUV. Spectrophotometric methods for the determination of zolmitriptan in bulk and pharmaceutical dosage forms. *J Adv Sci Res*. 2011;2:42–47.
22. Kumar B, Roy M, Kumar N, Kumar B, Tyagi PK, Khan K. Non-ionic surfactant vesicles of silymarin: formulation development and evaluation. *Int J Pharm Sci Rev Res*. 2014;29:1–5.
23. Fahmy AM, El-Setouhy DA, Ibrahim AB, Habib BA, Tayel SA, Bayoumi NA. Penetration enhancer-containing spanlastics (PECSs) for transdermal delivery of haloperidol: in vitro characterization, ex vivo permeation and in vivo biodistribution studies. *Drug Deliv*. 2018;25(1):12–22. doi:10.1080/10717544.2017.1410262

24. Zeng W, Li Q, Wan T, et al. Hyaluronic acid-coated spanlastics facilitate tacrolimus ocular delivery: mucoadhesion, precorneal retention, aqueous humor pharmacokinetics, and transcorneal permeability. *Colloids Surf B Biointerfaces*. 2016;141:28–35. doi:10.1016/j.colsurfb.2016.01.014
25. Sohrabi S, Haeri A, Mahboubi A, Mortazavi A, Dadashzadeh S. Chitosan gel-embedded moxifloxacin spanlastics: an efficient antimicrobial hybrid system for burn infection. *Int J Biol Macromol*. 2016;85:625–633. doi:10.1016/j.ijbiomac.2016.01.013
26. Jain S, Vyas SP. Mannosylated spanlastics as carrier adjuvant system for topical immunization. *J Pharm Pharmacol*. 2005;57:1177–1184. doi:10.1211/jpp.57.9.0012
27. Li Y, Zhang Q, Qi D, et al. Valproate ameliorates nitroglycerin-induced migraine in trigeminal nucleus caudalis in rats through inhibition of NF-κB. *J Headache Pain*. 2016;17:49. doi:10.1186/s10194-016-0631-z
28. Pradhan AA, Smith ML, McGuire B, Tarash I, Evans CJ, Charles A. Characterization of a novel model of chronic migraine. *Pain*. 2014;155:269–274. doi:10.1016/j.pain.2013.10.004
29. Cui Z, Chen L, Bei G, et al. Tetrandrine attenuates NF-kB activation in trigeminal ganglia by blocking calcium channel in a rat model of migraine. *J Medi Plants Res*. 2011;5(25):6032–6039.
30. Yao G, Man Y, Luo X, et al. Rizatriptan benzoate influences the endogenous pain modulatory system in a rat model of migraine. *Neural Regen Res*. 2012;7(2):131–135. doi:10.3969/j.issn.1673-5374.2012.02.009
31. Zhang Y, Huo M, Zhou J, Xie S. PKSolver: an add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. *Comput Methods Programs Biomed*. 2010;99:306–314. doi:10.1016/j.cmpb.2010.01.007
32. Worldwide MI. Dynamic light scattering, common terms defined. Inform White Paper. Malvern, UK: Malvern Instruments Limited; 2011:1–6.
33. Bini KB, Akhilesh D, Prabhakara P, Kamath JV. Development and characterization of non-ionic surfactant vesicles (spanlastics) for oral delivery of lornoxicam. *Int J Drug Dev Res*. 2012;4:147–154.
34. Greco R, Meazza C, Mangione AS, et al. Temporal profile of vascular changes induced by systemic nitroglycerine in the meningeal and cortical districts. *Cephalgia*. 2011;31:190–198. doi:10.1177/0333102410379887
35. Christiansen I, Versen HK, Olesen J, et al. Nitric oxide- induced headache may arise from extracerebral arteries as judged from tolerance to isosorbide-5 mononitrate. *Headache Pain*. 2008;9:215–220. doi:10.1007/s10194-008-0043-9
36. Jim B. Could glial activation be a factor in migraine? *Med Hypotheses*. 2009;72:255–257. doi:10.1016/j.mehy.2008.09.048
37. Monteith T, Goadsby PJ. Acute migraine therapy: new drugs and new approaches current treatment option. *Neurol*. 2011;13(1):1–14.
38. Arulmozhi DK, Veeranjaneyulu A, Bodlhamkar SL. Migraine:current concepts and emerging therapies. *Vasc Pharmacol*. 2005;43(3):176–187. doi:10.1016/j.vph.2005.07.001
39. Akbari V, Abedi D, Sadeghi- Aliabadi H. Release studies on ciprofloxacin loaded non-ionic surfactant vesicles. *Avicenna J Med Biotechnol*. 2015;7(2):69–75.
40. Khezri FA, Lakshmi CSR, Nargund SL, et al. Analgesic effect of zolmitriptan nanoparticles in experimental animal models. *IJIRMS*. 2018;3(7):2103–2107.
41. Christiansen I, Thomsen LL, Daugaard D, Ulrich V, Olesen J. Glyceryl trinitrate induces attacks of migraine without aura in sufferers of migraine with aura. *Cephalalgia*. 1999;19(7):660–667. doi:10.1046/j.1468-2982.1999.019007660.x
42. Olesen J. Nitric oxide-related drug targets in headache. *Neurotherapeutics*. 2010;7(2):183–190. doi:10.1016/j.nurt.2010.03.006
43. Bates EA, Nikai T, Brennan KC, et al. Sumatriptan alleviates nitroglycerin-induced mechanical and thermal allodynia in mice. *Cephalalgia*. 2010;30(2):170–178. doi:10.1111/j.1468-2982.2009.01864.x
44. Markovics A, Kormos V, Gaszner B, et al. Pituitary adenylate cyclase-activating polypeptide plays a key role in nitroglycerol-induced trigeminovascular activation in mice. *Neurobiol Dis*. 2012;45(1):633–644. doi:10.1016/j.nbd.2011.10.010
45. Bohotin C, Alexa T, Luca A, et al. The effects of intrathecal methylene blue and glyceryl trinitrate administration on orofacial pain in mice. *Int J Clin Neurosci Mental Health*. 2016;3(suppl.1):s17. doi:10.21035/ijcnmh