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Development and characterization of zolmitriptan loaded thiolated chitosan nanoparticles for intranasal drug delivery

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Abstract
The present study aimed to formulate thiolated chitosan nanoparticles of zolmitriptan for brain targeting via intranasal drug delivery. The thiolated chitosan was synthesized by reaction of thiogycolic acid and chitosan in presence of catalyst. The thiolated chitosan was characterized using FTIR and thermal studies. Drug loaded nanoparticles were prepared by ionic gelation of thiolated chitosan by using sodium tripolyphosphate. The prepared nanoparticles were characterized by FTIR spectroscopy to confirm the drug loading without any chemical interactions between the drug and excipients. The morphology and size of the nanoparticles were affirmed by using TEM analysis. The drug permeation of nanoparticles through nasal mucosa was evaluated by Franz diffusion cell for intranasal drug delivery.

Keywords: Thiolation, Nanoparticles, Intranasal drug delivery, Migraine

Introduction
Zolmitriptan (ZM) is a second generation triptan derivative which acts as serotonin 5-HT_1 receptor agonist and is primarily used in treatment of acute migraine in adults [1]. It directly and selectively constricts intracranial, extracerebial blood vessels and inhibits the release of the vasoactive neuropeptides from perivascular nerves to prevent neurogenic vasodilation and extravasation in the dura matter. Centrally, triptans also inhibit excitability of cells in the trigeminal nucleus caudalis via 5-HT_1 D agonism [2].
Due to hydrophilic nature zolmitriptan is well absorbed by oral and intranasal administration with approximately 40-42% bioavailability achieves peak plasma levels in 2–4 hours. The appearance of zolmitriptan's active metabolite (N-desmethyl zolmitriptan) in plasma following intranasal administration occurs at about 30 minutes after nasal administration. Thus first-pass metabolism in the liver is avoided with the nasal formulation; this active metabolite of zolmitriptan (ZM) may contribute to increase the in therapeutic efficacy of the drug as compared to oral delivery [3, 4]. Intranasal administration transports the drug directly to brain via olfactory nerve pathways. Intranasal route has been investigated to bypass blood brain barrier and to avoid systemic side effects for many drugs acting on central nervous system [5]. Thus intranasal administration offers a practical, noninvasive, and an alternative route of administration for rapid drug delivery to brain which may be effectively utilized to improve therapeutic efficacy of antimigraine drug like zolmitriptan.
Chitosan is a mucoadhesive biodegradable polymer and widely studied for the preparation of polymeric nanoparticles for the nose to brain drug delivery. It increases the permeability of the epithelial membrane by opening the tight junctions and facilitate paracellular transport across the nasal epithelial membrane. It also increases the residence time of the formulation over the olfactory epithelia by mucoadhesive forces. Thiolation of chitosan further improves the mucoadhesive potential as thiolated chitosan (TCH) tightly adhere to mucosal epithelia through covalent bonding with mucin gylcoprotiens via thiol-disulfide linkage [6, 7].
In the present study thiolated chitosan nanoparticles of Zomitriptan (ZM-TCH-NPs) were prepared by ionic gelation method [8]. The thiolated chitosan (TCH) was synthesized by reaction chitosan and thiogycolic acid in the presence of (1-ethyl-3-(3-dimethyl amino-propyl) carbodimide hydrochloride as catalyst and characterized by FTIR and DSC studies. Zolmitriptan loaded thiolated chitosan nanoparticles (ZM-TCH-NPs) were formulated and characterized for particle size, shape, zeta potential, entrapment efficiency, and FTIR spectroscopy. The nanoparticles were further studied for *ex- vivo* permeation through nasal mucosa using Franz diffusion cell.

Materials and methods

Drug and chemicals

Zolmitriptan was gifted by Alembic Pharmaceuticals Ltd., (Baroda), India. Chitosan (CH) was obtained as gift sample from CIFT, Kochi, India. Sodium tripoly phosphate (TPP), 1-ethyl-3-(3-dimethyl amino-propyl) carbodimide hydrochloride (EDAC) and thioglcolic acid (TGA) were purchased from sigma. Dialysis tubing (capacity approx. - 3.63 ml/cm) was purchased from Himedia and all other chemicals used are of analytical grade.

Synthesis of thiolated chitosan

Chitosan (500 mg) was dissolved in 50 ml of 1% acetic acid to get solution of 10 mg/ml. EDAC dissolved in 1ml deionized water was added to a final concentration of 125mM. To the above solution prepared and add 500 mg of TGA. Then pH of the medium was adjusted below 5 and the reaction mixture was incubated for 4h in dark at room temperature with constant stirring. For the isolation of unreacted polymer (TCH) from the reaction mixture, the polymer solution was dialyzed in tubings of cellulose membrane (molecular weight cut -off 12-14 KDa) for 3 days in dark against 5mM HCl, then twice against the same medium containing 1% NaCl to reduce the ionic interactions between the cationic polymer and the anionic sulfhydryl groups. Dialysis process was carried out in dark at 4°C for avoiding the oxidation of sulfhydryl groups. After dialysis, the polymer sample was lyophilized and the freeze dried polymer was used for further studies.

Characterization of thiolated chitosan

FTIR spectra of chitosan (CH) and TCH were carried on Fourier transforms infrared spectrophotometer using KBr method. The new amide bond formation and thiol group substitution in thiolated chitosan were confirmed by the presence of characteristic peaks in FTIR spectra. DSC thermogram of chitosan (CH) and lyophilized TCH, chitosan, were done by simultaneous TGA/DSC equipment (TA Instruments DSC SDTQ600), in the temperature range of 30°C – 400°C, at a heating rate of 10°C /min, with continuous purging of nitrogen gas (flow rate 100 ml/min) for examining the morphological changes, degradation steps in polymers [8].

Nanoparticles preparation

ZM-TCH-NPs were formulated by ionic gelation method using TPP as cross linking agent [9]. Nanoparticles were obtained as result of the drop wise addition of TPP solution to the aqueous solution of polymer (TCH) by continuous stirring, TCS to TPP weight ratio used was 3:1. As a result of ionic cross linking, a turbid solution was obtained, which was further stirred for half an hour. The resultant nanoparticles were separated by centrifugation at 13,000 rpm for 1h at 4◦C. The pellets were redispersed in water and lyophilized by using mannitol as cryoprotectant.

Characterization of formulated nanoparticles

Measurement of particle size and zeta potential

Average particle size (Z-average), polydispersity index (PDI) and zeta potential of the prepared nanoparticles were determined by dynamic light scattering analysis using Zetasizer. All the measurements were carried out by dispersing the nanoparticles in appropriate volume of deionised water at 25 °C.

Percent drug entrapment efficiency (%DEE)

The supernatant of formulations after centrifugation were collected and filtered. The amount of drug present was determined by UV- spectrophotometer. The Percentage drug entrapment efficiency (DEE) was calculated using formula:

$$\%\ DEE = \frac{Total\ amount\ of\ drug\ (W) - Free\ drug\ in\ supernatant(w)}{Total\ amount\ of\ drug\ (W)} \times 100$$

Transmission electron microscopy (TEM)

The size and morphological characteristics of the optimized ZM-TCH-NPs were further confirmed by TEM. Formulated nanoparticle suspension was diluted in deionised water and sonicated for 5 min to produce disaggregation of the particles. One drop of the sample was stained with 2% phosphotungstic acid, deposited on a 300 mesh formvar coated grid and then examined under HRTEM equipment (TECHNAIS TWIN) operated at 200 kV.

Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopic analysis of polymer (TCH), pure drug (ZM) and lyophilized ZM-TCH-NPs were done by Fourier transform infrared spectrophotometer (Perkin Elmer, USA) using KBr method and scanned in the frequency range of 4000 to 400 cm^{-2}.

Ex- vivo drug permeation study using Franz diffusion cell:

To study the drug permeation behavior through nasal mucosa, *ex- vivo* study using Goat nasal mucosa (obtained from Slaughter house) was performed using Franz diffusion cell [10, 11]. Nasal mucosa was washed with phosphate buffer pH 6.4 and size of contact area 1.55 cm^2 was mounted on the receptor compartment of the Franz diffusion cell (diameter 10mm, 15ml volume), with dermal face in contact with the phosphate buffer (pH 6.4). Two experimental sets in triplicate were performed keeping the temperature 37±0.5 °C, at speed of 100 rpm. The ZM-TCH-NPs were resuspended in 2ml phosphate buffer pH 6.4 and placed on the surface of nasal mucosa. 2ml of sample was withdrawn from receptor compartment at 0min, 15min, 30min, 1h, 2h, 4h, 6h, 8h, 16h, and 24 hr and replaced with 2ml of fresh phosphate buffer to maintain sink conditions. The pure drug aqueous solution was taken as control group. Samples were analyzed by UV- spectroscopy at 220 nm for the estimation of amount of drug permeated with time.

Results and discussion

Characterization of thiolated chitosan

FTIR spectra of chitosan and TCH are shown in Figure 1and 2 respectively. In the spectrum of chitosan , the characteristic peaks are at 3431cm^{-1} ($\upsilon_{o\text{-}H}$ and $\upsilon_{N\text{-}H}$), 2925 cm^{-1} ($\upsilon_{C\text{-}H}$), 1645 cm^{-1}, 1517 cm^{-1} ($\delta_{o\text{-}H}$), 1078 cm^{-1} ($\upsilon_{C\text{-}N}$), 617 cm^{-1} (δ_{NH}), 1376 cm^{-1} ($\delta_{C\text{-}H}$), 1248 cm^{-1} ($\delta_{o\text{-}H}$), 1154 cm^{-1} ($\delta_{C\text{-}O\text{-}C}$). In TCH the amide bond formation occurs between amino groups and carboxyl groups, so characteristic peaks of amide bonds are 1527 cm^{-1} (amide I band), 1638 cm^{-1} (amide II band). The peak at 1248 cm^{-1} appears due to thiol substitution. All other peaks are similar to chitosan. Chitosan to degrade near about 300 °C and has broad degradation temperature range (Figure 3). Degradation involves dehydration, deacetylation and chain scission [8]. The degradation first started with loss of water. TCS degradation start at lower temperature (150 °C) compared to chitosan due presence of weaker amide bond (-NCO-CH_2-SH) formed in thiolation (Figure 4).

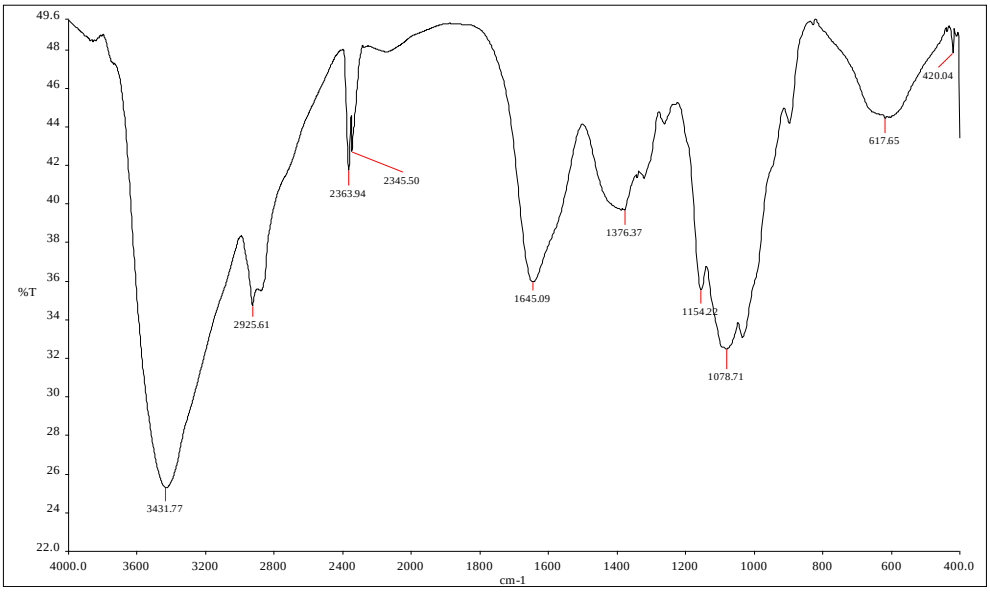

Fig 1: FTIR spectra of Chitosan

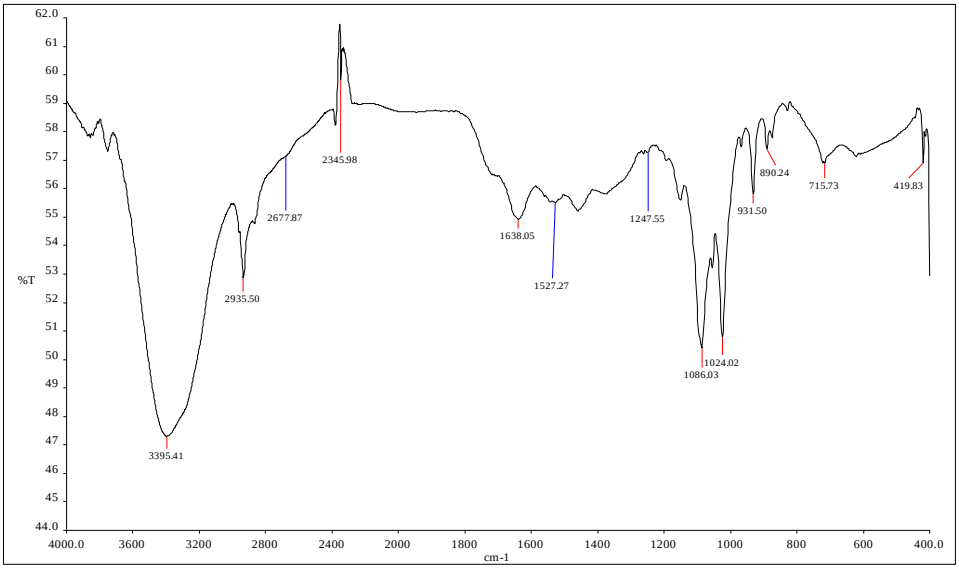

Fig 2: FTIR spectra of TCH

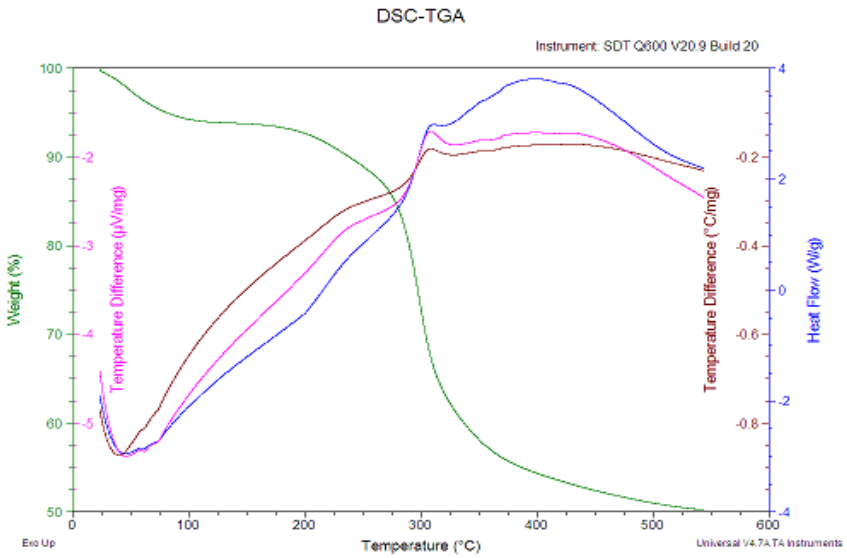

Fig 3: DSC-TGA thermogram of Chitosan (CH)

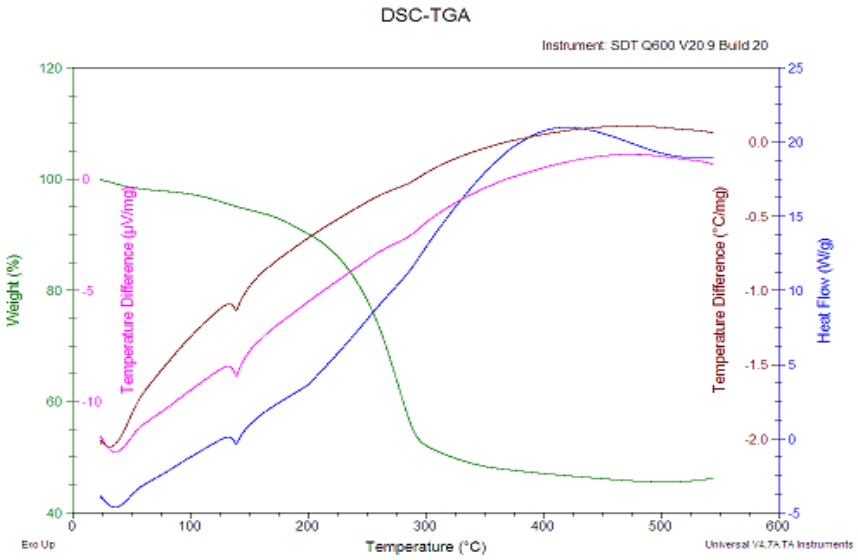

Fig 4: DSC-TGA thermogram of TCH

Particle size, zeta potential and drug encapsulation efficiency

The average particle size of the ZM-TCH-NPs was found to be 238.2nm having zeta potential value +27.2mV. Drug encapsulation efficiency of nanoparticles was found to be 85.05%.

Transmission electron microscopy (TEM)

ZM-TCH-NPs observed under HRTEM, revealed the particles to be spherical in shape. The particles size observed under TEM image are in range 50 to 90 nm (Figure 5). The difference in size of particles measured from Zetasizer explained due to removal hydrodynamic layers covering the particles, during the sample preparation for TEM analysis, so leads to decrease in size.

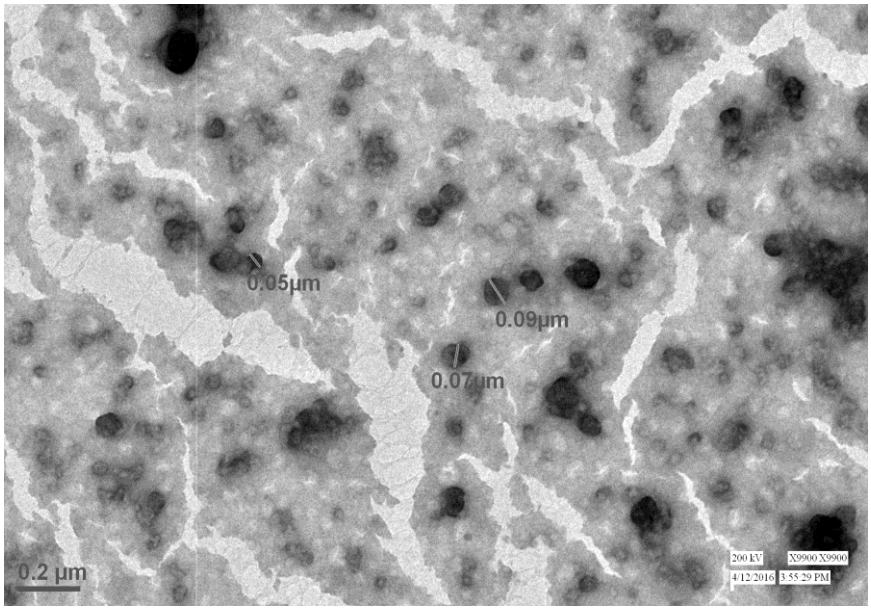

Fig 5: TEM image of ZM-TCH-NPs

FTIR spectroscopy

FTIR spectra of Zolmitriptan (ZM), TCH and ZM-TCH-NPs are shown in the spectrum of chitosan, the characteristic peaks are at 3431cm-1 (υo-H and υN-H), 2925 cm-1 (υC-H), 1645 cm-1, 1517 cm-1 (δo-H), 1078 cm-1 (υC-N), 617 cm-1 (δNH), 1376 cm-1 (δC-H), 1248 cm-1 (δo-H), 1154 cm-1 (δC-O-C). TCH Shows all the peaks of chitosan and additional peaks due to new amide bond formation occurs between amino groups and carboxyl groups, so characteristic peak of amide bonds are 1527 cm^{-1} (amide I band), 1638 cm^{-1} (amide II band) and the peak appears at 1248 cm^{-1} due to thiol substitution. The FTIR spectrum of ZM-TCH-NPs represented all the characteristic peaks of the drug as well as excipients, thereby confirming the absence of any chemical interaction between them [8].

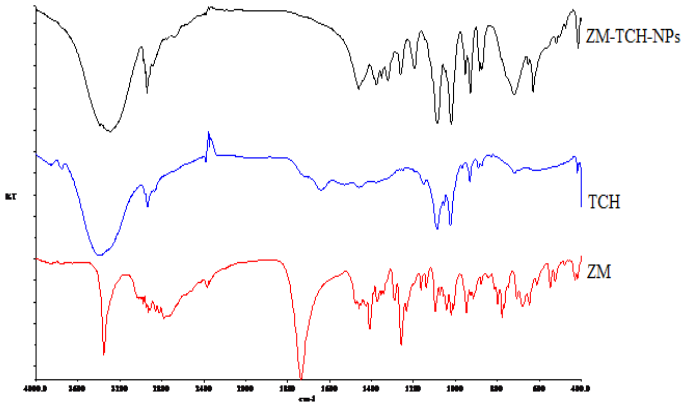

Fig. 6: FTIR spectra of ZM, TCH and ZM-TCH-NPs.

Ex-vivo drug permeation studies

Goat nasal mucosa was selected as biological membrane for permeation studies using Franz diffusion cell. The ZM-CH-NPs were compared with ZM aqueous suspension for permeation across the nasal mucosa. The amount of drug permeated at different time intervals are given in table. Drug permeated from NPs showed sustained permeation up to 24 hours whereas pure drug showed rapid drug permeation up to 90% in 6 hours across nasal mucosa. The graph of % drug permeated versus time was plotted (Figure 7), and the regression plot was used to calculate the permeation constant given by the slope of the regression line and flux (J) value calculated for ZM-TCH-NPs and GT solution are 8.49 and 41.18 respectively shown in figure 8.

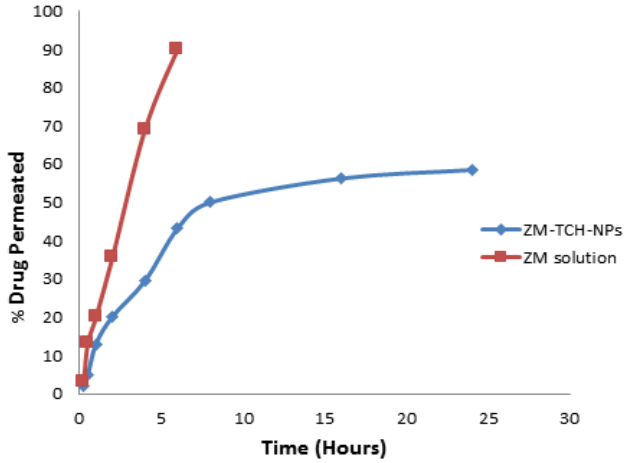

Fig 7:*Ex- vivo* drug permeation study of pure drug (ZM) and optimized formulation (ZM TCH-NPs)

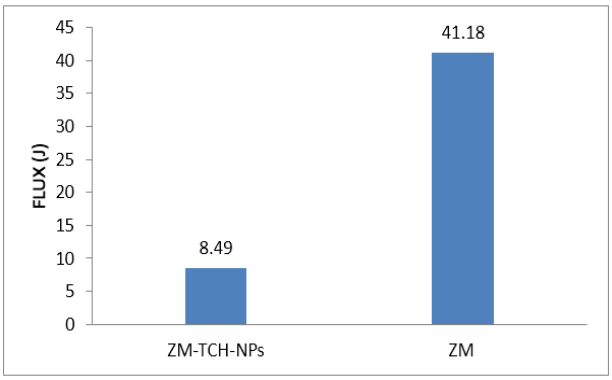

Fig 8: Bar graph showing flux of pure drug (ZM) aqueous solution and optimized formulation (ZM-TCH-NPs)

Conclusions

The mucoadhesive polymer, thiolated chitosan (TCH) was synthesized. The thiol substitution in chitosan was confirmed by FTIR, and DSC studies. The drug loaded ZM nanoparticles were characterized using FTIR and TEM studies. The prepared NPs possess a size range 50-90 nm and spherical in shape. By Zeta potential measurement the ZM-TCH-NPs was found to be stable and having positive surface charge. *Ex vivo* drug permeation studies reveals controlled drug release up to 24 hours. Thus the thiolated chitosan based zolmitriptan nanoparticles severves a potential intranasal drug delivery tool for brain targeting in migraine.

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