Preparation and the *in Vitro* Evaluation of Nanoemulsion System for the Transdermal Delivery of Granisetron Hydrochloride

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The objective of this study was to develop and evaluate nanoemulsion system for transdermal delivery of granisetron hydrochloride. Pseudo-ternary phase diagram was constructed to ascertain the concentration range of components of nanoemulsion composed of isopropyl myristate (IPM) as an oil phase, tween 85 as surfactant, ethanol as cosurfactant, water as aqueous phase. The effects of the content of IPM as an oil phase and *n*-methyl pyrrolidone (NMP) as transdermal enhancer on rat skin permeation of granisetron hydrochloride nanoemulsion were studied *in vitro*. The results showed that the mean particle size of nanoemulsion ranged from 50.4±1.5 to 82.4±0.9 nm with homogeneous size distribution. The resulted optimum formulation composed of 2.5% granisetron hydrochloride, 4% IPM, 40% tween 85/ethanol (1 : 1) and 10% NMP showed that the skin permeation rate was the highest (85.39±2.90 μg/cm^2/h) and enhancement of drug permeability was 4.1-folde for transdermal delivery of granisetron hydrochloridein comparison with the control group (20% of tween 85 and 20% of ethanol micelle solution containing 2.5% of granisetron hydrochloride without IPM), and cumulative permeation amount was the highest (891.8±2.86 μg/cm^2) with the shortest lag time (0.11±0.02 h) and was stable for at least 12 months. Therefore, the nanoemulsion system developed in this study offers a promising vehicle for the transdermal delivery system of granisetron hydrochloride, which may be as effective as oral or intravenous dosage forms and avoid some difficulties associated with these dosage forms.

Key words granisetron hydrochloride; nanoemulsion; transdermal drug delivery; isopropyl myristate; *N*-methyl pyrrolidone; skin permeation

Nausea and vomiting are the commonest side effects related to cancer cytotoxic chemotherapy and radiation therapy, which impact on patients' quality of life and optimal therapy.[1—4] Therefore, prevention of nausea and vomiting is very important to improve patient compliance and therapeutic effect. Marked benefit in antiemetic therapy has been observed since the introduction of the 5-HT3 receptor antagonists such as granisetron hydrochloride.[3] Due to its well tolerate and high pharmacological activity, granisetron hydrochloride regarded as the first-line antiemetic drugs in clinical, has been used widely in antiemetic therapy.[4] In clinical, it is mainly available for injection and oral dosage forms as tablets or oral solution. For oral route, it produces severe first-pass effect and leads to some difficulties for cancer patients who are unable or unwilling to take an oral dosage forms. On the other hand, intravenous dosage form results in injure and ache for patients.[5] However, significant advantages of transdermal delivery of granisetron hydrochloride in solving these problems could be achieved.

At present, tansdermal dosage forms include patch,[6,7] microemulsion,[8—18] gels,[19—21] cataplasm[5] and Aerosol.[22,23] Microemulsion consisted of oil and aqueous phase, surfactant and cosurfactant, has unique advantages such as good thermodynamic stability, ease of manufacturing and enhancement of the skin permeation of many drugs.[9—15] Recently, microemulsion has been developed as a potential vehicle for tansdermal delivery of drugs.[24]

The aim of this study was to prepare an oil/water (o/w) nanoemulsion system for transdermal delivery of granisetron hydrochloride and evaluate its tansdermal behavior through rat skin to find out the optimum formulation with the highest skin permeability.

Experimental

Materials Granisetron hydrochloride was obtained from Sichuan Haikang Pharmaceutical Co., Ltd. (Sichuan, P.R. China). Isopropyl myristate (IPM), *n*-methyl pyrrolidone (NMP), polyoxyethylene sorbitan trioleate (Tween 85), ethanol and other reagents (Analytical grade) were purchased from Chengdu Chemical Regent Co., Ltd. (Chengdu, P.R. China). Methanol used (HPLC grade) was purchased from Jiangsu Hanbang Co., Ltd. (Jiangsu, P.R. China). Water was purified by double distillation in a glass apparatus.

Construction of Pseudo-Ternary Phase Diagram On the basis of preliminary experiment, in this study, we chose IPM as oil phase, tween 85 as surfactant, ethanol as cosurfactant, water as aqueous phase and NMP as transdermal enhancer. Surfactant and cosurfactant (K_m) were mixed in different mass ratios (3 : 1, 1 : 1, 1 : 3). Pseudo-ternary phase diagram composed of oil, K_m and aqueous phase was developed using water titration method at room temperature to ascertain the concentration ranges of components. The mixtures of oil and K_m at different mass ratios from 1 : 9 to 1 : 0.1 were added with aqueous phase dropwise under gentle agitation to observe transparent and easily flowable o/w nanoemulsion.

According to the above nanoemulsion regions in the pseudo-ternary phase diagram, appropriate concentrations of oil, surfactant and cosurfactant were selected and used to prepare granisetron hydrochloride nanoemulsion. Furthermore, the effects of the content of isopropyl myristate (IPM) and *n*-methyl pyrrolidone (NMP) on rat skin permeation of granisetron hydrochloride nanoemulsion were studied *in vitro*.

Measurement of Particle Size The mean particle size and polydispersity index of granisetron hydrochloride nanoemulsion were measured by Malvern ZEN3600 (Malvern Instruments, U.K.).

***In Vitro* Skin Permeation Study** Rats weighting 200±20 g used in this study were obtained from the Laboratory Animal Center of Luzhou Medical College and approved by the Luzhou Medical College animal ethical experimentation committee (Sichuan, P.R. China). After hair in the abdominal region was removed carefully with an electric clipper, these rats were sacrificed immediately and the full skin was excised from the abdominal region. Then subcutaneous tissue and fat were removed surgically. The skin was washed with 0.9% saline, and then stored at 4 °C and used within 24 h.

In vitro skin permeation study was performed on a Franze diffusion cells (RYJ-6B) fitted with an effective diffusion area of 2.8 cm^2 and 6.5 ml of re-

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ceptor compartment capacity using excised rat skins at 37±0.5 °C. The receptor compartment was filled with 0.9% saline, which was magnetically stirred at 400 rpm throughout the experiment. After the granisetron hydrochloride nanoemulsion samples (0.2 g) were mounted on the epidermal surface of the excised rat skin, at regular time interval, the receiver solution was completely withdrawn and filtered with a 0.22 μm membrane filter to determinate concentration of granisetron hydrochloride by HPLC analytical method as described below. At the same time, fresh 0.9% saline (6.5 ml, 37±0.5 °C) was dispensed into the receptor compartment to maintain sink conditions after each sample was collected.

Drug Quantification The concentration of granisetron hydrochloride in the receptor medium was assayed with a modified reverse phase HPLC method reported previously.[25] Phenomenex LUNA C18 column (150 mm×4.6 mm, 5 μm particle sizes) (Phenomenex, U.S.A.) with a guard column (Phenomenex C18, 4.0 mm×3.0 mm) was selected and used. The mobile phase was composed of methanol/0.02 M phosphate buffer (pH 4.0) (40 : 60, v/v), running at a flow rate of 1 ml/min with ultraviolet (UV) detection at 303 nm.

The Stability of Nanoemulsion The acceleration stability test and long-term stability test were carried out according to the Technical Standard of Drug Stability Test (Chinese Pharmacopoeia 2005, appendix XIX C). Granisetron hydrochloride nanoemulsion samples prepared by the optimum formulation were stored at 40±2 °C, RH 60±10% for 6 months in the case of the accelerate stability test, and sampled at months 1, 2, 3 and 6. In the long-term stability test, Granisetron hydrochloride nanoemulsion samples were preserved at 25±2 °C for 12 months, and sampled at months 0, 3, 6, 9 and 12. The appearance, content and related substances as markers of stability were determined according to the methods described above.

Data Analysis *In vitro* skin permeation study, the cumulative amount of the drug permeation per unit of rat skin surface area (Qt) was determined by the following equation:

$$Qt = \frac{VrCt + \sum VsCi}{A}$$

Where Ct is the drug concentration of the receiver solution at each sampling time, Ci is the drug concentration of the ith sample, and Vr and Vs represent the volumes of the receiver solution and the sample solution, respectively, A is the effective diffusion area of skin surface. The curve of Qt *versus* time was analyzed by linear regression to obtain linear regression equation: $Qt=kt\pm b$, where k is the slop of the curve. The skin permeation rate at steady-state (Js, μg/cm^2/h) is calculated by the equation: $Js=k$. Lag time (t) is defined as the first time of detected drug and calculated by the equation: $t=\pm b/k$ ($Qt=0$). Statistical analysis was performed by the Student's t-test and a significance level of less than 0.05 was considered statistically significant.

Results and Discussion

Phase Diagram Study and Nanoemulsion Formation When we investigate pharmaceutical formulation, important criteria of components selection are that these components can be pharmaceutically acceptable. Based on the preliminary experimental results, IPM was chosen as oil phase and the maximum area of o/w nanoemulsion was observed, which was consistent with previous results.[24] Since a great number of surfactants may cause skin irritation, a major determining factor in choosing a surfactant is safety firstly. Non-ionic surfactants are less toxic than ionic surfactants. In general, the formation of o/w nanoemulsion requires the hydrophilic lipophilic balance (HLB) value (≥10). It was well known that the HLB value of IPM (11.1) was similar to that of Tween 85 (11.0). Kloet *et al.* reported that the emulsifying effect was the best when the HLB value of selected surfactant was equal to that of oil phase.[26] So, Tween 85 was selected as surfactant. In this study, an ethanol was chosen as cosurfactant, which was necessary to maintain stable o/w nanoemulsion.

The pseudo-ternary phase diagram of IPM as an oil phase, tween 85 as surfactant, ethanol as cosurfactant, water as aqueous phase was developed to ascertain the components concentration range for the formation of nanoemulsion. It was found that o/w nanoemulsion created with the three systems (Figs. 1a—c) was thermodynamically stable, optically

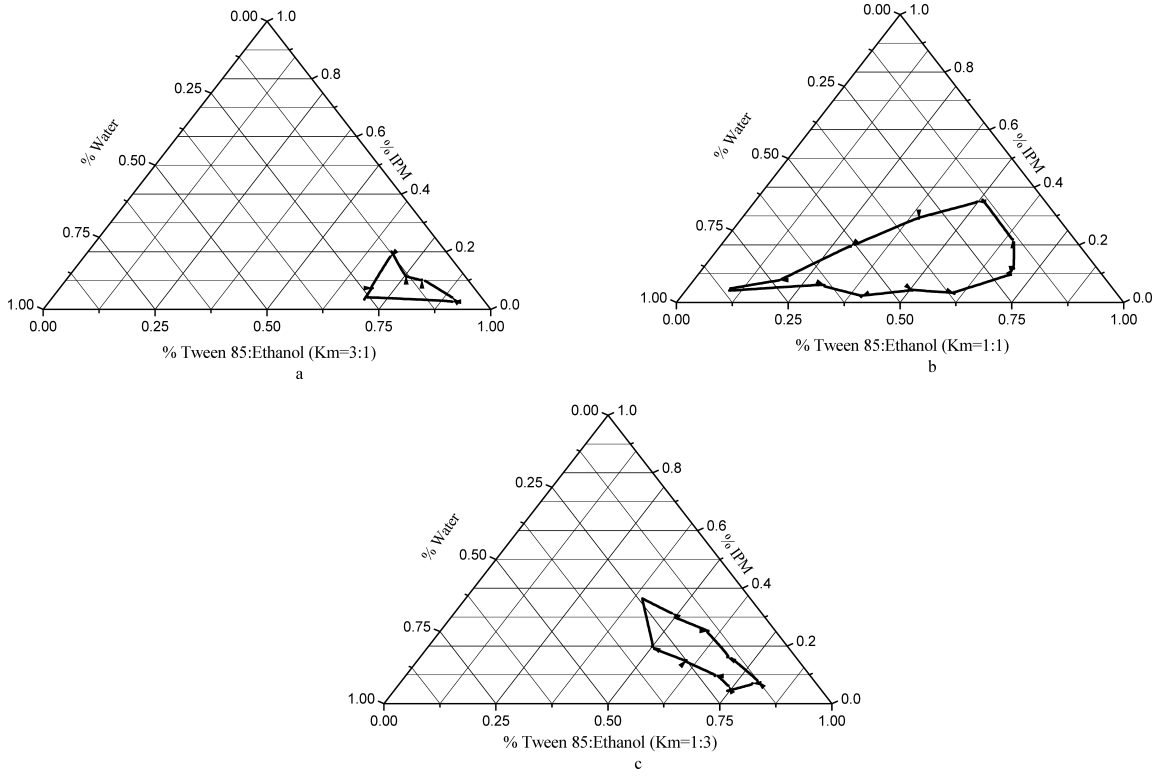

Fig. 1. Pseudo-Ternary Phase Diagram of Nanoemulsion Composed of Oil Phase (IPM), Surfactant (Tween 85), Cosurfactant (Ethanol) and Water

Table 1. Mean Particle Size, Polydispersity Index (PI) and Permeation Parameters of Granisetron Hydrochloride Nanoemulsion Composed of the Different Content of IPM[a)]

Content of IPM (%)	Mean size (nm)	PI	*Js* (μg/cm^2/h)	Lag time (h)	Cumulative amount (μg/cm^2)
0 (control)[b)]	—	—	20.99±4.58	0.50±0.03	245.1±4.91
4.0	52.4±1.6	0.29±0.01	69.57±5.97	0.23±0.03	780.7±3.85
8.0	64.8±1.4	0.27±0.02	63.04±4.31	0.31±0.05	740.5±4.75
12.0	82.4±0.9	0.26±0.02	51.22±2.89	0.44±0.04	600.2±3.74

a) The values are the means of three samples and standard deviation. *b*) Twenty percent of tween 85 and 20% of ethanol micelle solution containing 2.5% of granisetron hydrochloride without IPM.

transparent and single phase of liquid solution. The effect of the weight ration of surfactant and cosurfactant (K_m) on the area of o/w nanoemulsion region was compared. It can be seen from Figs. 1a—b that the area of o/w nanoemulsion region increased with the decreasing ratio of K_m from 3 : 1 to 1 : 1. The maximum area of o/w nanoemulsion region was observed when K_m was 1 : 1, while a very narrow area of o/w nanoemulsion region was obtained at K_m 3 : 1. This reason was that ethanol was a polar solvent with the tendency to highly incorporate into aqueous phase and was capable of solubilizing high water content.[27)] Therefore, the relatively lower ethanol concentration decreased the hydrophilicity of the mix-surfactant resulted in small area of o/w nanoemulsion region. In contrast, at K_m 1 : 3, the low concentration of surfactant reduced the amount of micelle, resulted in low solubilization capacity of nanoemulsion, which a small area of nanoemulsion region was observed.

Effect of the Content of Oil Phase on Skin Permeation In the present study, on the basis of nanoemulsion regions in the pseudo-ternary phase diagram, when the content of K_m (1 : 1) and granisetron hydrochloride was fixed at 40% and 2.5%, respectively, appropriate content of oil was selected and used to prepare granisetron hydrochloride nanoemulsion. The effect of the content of IPM on nanoemulsion characterization and skin permeation was investigated. These results were shown in Table 1 and Fig. 2, respectively, which showed the effect of the content of IPM ranged from 4.0 to 12.0% on the skin permeation behavior of granisetron hydrochloride. From the Fig. 2 and Table 1, the cumulative permeation amount of granisetron hydrochloride at 12 h were the highest when 4% of IPM was used and the skin permeation rate increased 3.3-fold in comparison with control group (20% of tween 85 and 20% of ethanol micelle solution containing 2.5% of granisetron hydrochloride without IPM), which demonstrated that nanoemulsion as a transdermal permeation carrier had excellent penetrable ability through skin. *In vitro* skin permeation study, the skin permeation rate and cumulative amount of granisetron hydrochloride increased with the decreasing IPM content; in contrast, lag time was reduced. Among the formulations containing different IPM content, a significant difference in lag time, cumulative amount and the skin permeation rate was observed ($p<0.025$). One reason was that water in the nanoemulsion system could hydrate skin to promote drug channels wide, which was consistent with the previous results reported.[16,28)] Other was that the drug in nanoemulsion system could penetrate skin in the form of nanoemulsion droplet. From Table 1, the mean particle size of nanoemulsion increased with the increasing concentration of IPM, which could be attributed to the increase

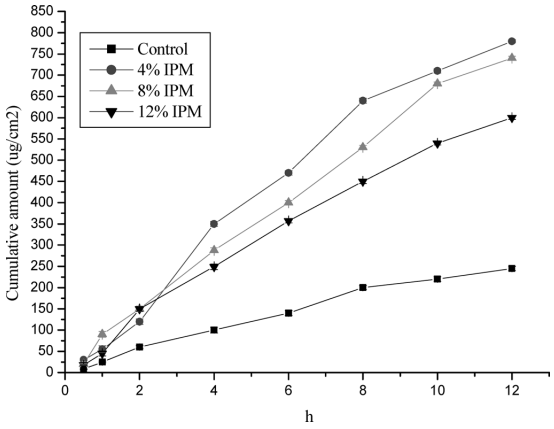

Fig. 2. *In Vitro* Permeation–Time Curve through the Rat Skin from Granisetron Hydrochloride Nanoemulsion Containing Different Content of IPM

Control group represents 20% of tween 85 and 20% of ethanol micelle solution containing 2.5% of granisetron hydrochloride without IPM. These values are the means of three samples and standard deviation.

of oil drop of nanoemulsion by further titration of the oil. The result was accordance with previous study with nanoemulsion system where the mean particle sizes of nanoemulsion containing 5% and 15% IPM were 37.0 and 61.9 nm, respectively.[16)]

Effect of the Content of NMP on Skin Permeation Permeation enhancers are commonly used in the formulation of a transdermal drug delivery system for achieving the desired drug penetration rate. NMP is well known to be safe and it has been used to increase the skin permeation of a large number of drugs. In this study, when the IPM content was chosen as 4%, and the content of K_m (1 : 1) and granisetron hydrochloride was fixed at 40% and 2.5%, respectively, the effect of different content of NMP in the nanoemulsion system on the skin permeation of granisetron hydrochloride was studied and these results were shown in Table 2 and Fig. 3. It was found that the cumulative permeation amount ranged from 795.0±3.89 to 891.8±2.86 μg/cm^2, *Js* ranged from 71.14±3.54 to 85.39±2.90 μg/cm^2/h and lag time ranged from 0.11±0.02 to 0.25±0.01 h demonstrated that the permeation parameters of granisetron hydrochloride nanoemulsion were markedly affected by the content of NMP in the nanoemulsion system. For granisetron nanoemulsion containing NMP, there was a significant difference in lag time, cumulative amount and the skin permeation rate in comparison with the control group (nanoemulsion without NMP) ($p<0.025$), while no markedly change in the skin permeation parameters between nanoemulsion contain-

Table 2. Mean Particle Size, Polydispersity Index (PI) and Permeation Parameters of Granisetron Hydrochloride Nanoemulsion Composed of the Different Content of NMP[a)]

Content of NMP (%)	Mean size (nm)	PI	*Js* (μg/cm^2/h)	Lag time (h)	Cumulative amount (μg/cm^2)
0 (control)[b)]	50.4±1.5	0.28±0.01	71.14±3.54	0.25±0.01	795.0±3.89
5.0	47.5±1.6	0.26±0.01	76.16±4.21	0.15±0.03	850.4±4.21
10.0	48.3±1.7	0.27±0.02	85.39±2.90	0.11±0.02	891.8±2.86
15.0	47.4±1.4	0.31±0.01	84.47±4.75	0.14±0.02	889.1±2.24

a) The values are the means of three samples and standard deviation. *b*) Control group represents the nanoemulsion containing 2.5% of granisetron hydrochloride without NMP.

Table 3. The Results of the Accelerate Stability Test (Mean±S.D., n=3)[a)]

Items	Time (months)					
	0	1	2	3	6	*p*-Value[b)]
The appearance	Clarity and no creaming	Clarity and no creaming	Clarity and no creaming	Clarity and no creaming	Clarity and no creaming	—
The content (%)	99.34±0.40	99.61±0.31	99.20±0.46	99.21±0.55	99.48±0.44	0.73
The related substances (%)	0.17±0.01	0.14±0.01	0.19±0.02	0.18±0.01	0.19±0.01	0.10

a) Formulation of microspheres: 2.5% granisetron hydrochloride, 4% IPM, 40% tween 85/ethanol (1 : 1) and 10% NMP. *b*) The significant level of difference was defined as ≤0.05.

Table 4. The Results of the Long-Term Stability Test (Mean±S.D., n=3)[a)]

Items	Time (months)					
	0	3	6	9	12	*p*-Value[b)]
The appearance	Clarity and no creaming	Clarity and no creaming	Clarity and no creaming	Clarity and no creaming	Clarity and no creaming	—
The content (%)	99.34±0.40	99.58±0.31	99.49±0.38	99.71±0.45	99.61±0.40	0.80
The related substances (%)	0.17±0.01	0.13±0.01	0.17±0.01	0.11±0.01	0.16±0.01	0.31

a) Formulation of microspheres: 2.5% granisetron hydrochloride, 4% IPM, 40% tween 85/ethanol (1 : 1) and 10% NMP. *b*) The significant level of difference was defined as ≤0.05.

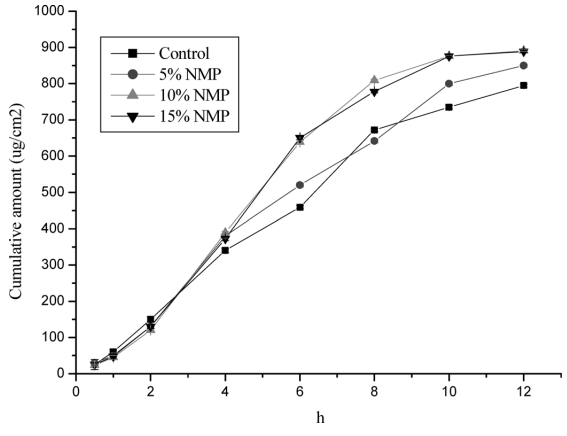

Fig. 3. *In Vitro* Permeation–Time Curve through the Rat Skin from Granisetron Hydrochloride Nanoemulsion Containing Different Content of NMP

Control group represents the nanoemulsion containing 2.5% of granisetron hydrochloride without NMP. The values are the means of three samples and standard deviation.

ing 10.0% NMP and 15.0% NMP was observed (p>0.05). These results were consistent with previous study reported that,[24,29,30)] the permeation effect of nanoemulsion containing hydrophobic or hydrophilic drugs was markedly enhanced in the existence of NMP in the nanoemulsion system, in comparison with control group (without NMP). In other words, these results indicated that NMP was a more effective transdermal enhancer. As a partition enhancer in the nanoemulsion system, NMP could increase the concentration of granisetron hydrochloride in the aqueous phase, making it improve the permeability of skin.[24)] In addition, data from Fig. 3 indicates that granisetron hydrochloride nanoemulsion penetrates rat skin *in vitro* by passive diffusion. Therefore, the skin permeation rate (*Js*) decreased with the decrease of drug concentration in the donor compartment at the latter half (6—12 h). If the drug in nanoemulsion system penetrates skin in the form of nanoemulsion droplet, the mechanisms of enhancement effect of NMP on excise rat skin penetration mainly include ① increasing the thermodynamic activity in the vehicle, ② fluidization of the lipid in the stratum corneum, reducing the diffusional resistance; ③ increasing drug solubility in the skin.[31)] Therefore, according to cumulative amount, the skin permeation rate and lag time as marker, we found out the optimum formulation composed of 2.5% granisetron hydrochloride, 4% IPM, 40% tween 85/ethanol (1 : 1) and 10% NMP.

The Stability of Nanoemulsion It was known that characterization of pharmaceutical formulation stability was a

key step in the design of safe, stable and effective drugs. Thus, both the acceleration stability test and long-term stability test were carried out to study on the stability of granisetron hydrochloride nanoemulsion (Tables 3, 4). The results of the accelerating stability test and long-term stability test showed no marked change in the appearance, content and related substances as indexes ($n=3$; $p>0.05$). Therefore, the granisetron hydrochloride nanoemulsion was stable for at least 12 months at room temperature.

Conclusion

In the present study, an o/w nanoemulsion system for transdermal delivery of granisetron hydrochloride using NMP as transdermal enhancer was developed successfully and the effect of the content of IPM and NMP on the skin permeation behavior of granisetron hydrochloride was evaluated to find out the desirable nanoemulsion formulation with the highest skin permeability. Although the pharmacokinetics has not been studied in animal and clinical experiment, from the results *in vitro*, we believe that it is promising to reach pharmacologically effective concentration of granisetron with use of new topical formulation. Therefore, further studies are needed to confirm this possibility.

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