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Preparation and pharmacokinetic study of mebendazole complex with HP-beta-cyclodextrin

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Mebendazole, approved by the Food and Drug Administration (FDA) in 1974 to expel intestinal parasite infections, was found to exhibit multiple anti-cancer activities. However, due to its poor water solubility ($0.33 \pm 0.02 \mu\text{g/mL}$), its clinical applications were greatly limited. Scientists at Wenzhou-Kean University have developed a formulation that could increase its water solubility as much as 18,333 times to reach 6.05 mg/mL as the best result to date. Through the complexation with HP-beta-cyclodextrin, 80% of mebendazole in the complex was released *in vitro* in 5 min, and only 20% of mebendazole was released under the same conditions for the pure drug, in a *in vivo* pharmacokinetic study conducted on dogs with a dose of 5 mg/kg . The C_{max} of mebendazole was increased from $8.96 \pm 0.15 \mu\text{g/mL}$ to $17.34 \pm 2.02 \mu\text{g/mL}$. T_{max} of mebendazole was shortened from $12.00 \pm 0.50 \text{ h}$ to $10 \pm 0.50 \text{ h}$. The half life time was prolonged from $5.81 \pm 0.36 \text{ h}$ to $10.01 \pm 2.07 \text{ h}$; the AUC_{0-24} was increased from $151.32 \pm 5.92 \mu\text{g}\cdot\text{h/mL}$ to $289.02 \pm 15.83 \mu\text{g}\cdot\text{h/mL}$; and the bioavailability was improved by 91%, indicating that this complex can be pushed to the next step as an anti-tumor agent for its clinic practice.

KEYWORDS

mebendazole, HP-beta-cyclodextrin, inclusion complex, complex preparation, *in vitro* and *in vivo* pharmacokinetic study

Introduction

Mebendazole is a synthetic benzimidazole carbamate that was approved by the Food and Drug Administration (FDA) in 1974 to expel intestinal parasite infections, hinder the formation of the microtubule system of parasite cells, make the cell cycle stop in the G2/M phase, and affect cell mitosis. Additionally, it inhibits the parasite's ability to uptake glucose, causes the glycogen stored in the parasite's body to deplete, and reduces the formation of adenosine triphosphate, ultimately interfering with energy metabolism and rendering the parasite un-survivable (1). Mebendazole is effective against soil-transmitted helminth infections in humans (2) caused by at least one type of nematodes (roundworms, hookworms, or whipworms).

Recently, mebendazole was demonstrated to exhibit preclinical efficacy against multiple cancers, including glioblastoma multiforme, medulloblastoma, and colon, breast, pancreatic, and thyroid cancers (3). It was considered as an excellent candidate for the treatment of brain tumors as a monotherapy and in combination with other treatments (4, 5). However, its poor water

solubility ($0.33 \pm 0.02 \mu\text{g/mL}$) hampered its performance and limited its clinical applications (6). Cancer drugs administered through IP or IV should have water solubility in the range of 5 mg/mL to 10 mg/mL to achieve the necessary therapeutic dose, and as an oral solid dosage formulation, mebendazole was used to treat advanced or metastatic gastrointestinal cancer with the dose of up to 4 g/day in the phase 2 clinic trial (3, 7). Patients with refractory gastrointestinal cancer were treated with individualized, dose-adjusted mebendazole. Therefore, there is an urgent need to find ways of increasing mebendazole's water solubility.

Many methods such as particle size reduction (8), solid dispersion (9–18), nanosuspensions (19–24) and salt formation (25–28) have been used to improve mebendazole's water solubility, and the results were not significant, more important, most of these methods involved the usage of polymers, which could be challenge for product's quality control and scaled up. Based on this consideration, cyclodextrins are the ideal drug carriers for increasing mebendazole's water solubility. The inclusion complexes of mebendazole with cyclodextrins such as α , β , γ , β -sulfated and HP- β -cyclodextrins showed an increase in water solubility ranging from 16 to 31 times (29, 30), when heated with HP- β -cyclodextrin in the presence of citric or tartaric acid at 95°C for 60 min. Mebendazole's water solubility was increased to 0.68 mg/mL (31, 32), and the formulation of β -cyclodextrin, chitosan-based microcrystals, polyvinyl alcohol and polysorbate 80-based nanoparticles could only improve mebendazole's solubility four times (33). Complexes with β -cyclodextrins and permethyl- β -cyclodextrin could increase the solubility by 35 times and 4,700 times, respectively (34). It was reported that solid dispersion could increase mebendazole's solubility 15,982 times (35); however, the solubility was measured in an acidic aqueous solution (0.1 M HCl), and therefore, the solubility result is not real.

Overall, although great efforts were made to increase mebendazole's water solubility, a significant increase in water solubility was not achieved. In this communication, we report our updated results from a study of mebendazole's water solubility through complexation with HP- β -cyclodextrin. As shown in Figure 1, the water solubility of mebendazole increased 18,333 times to reach 6.05 mg/mL, which is the best result to date. Meanwhile, the pharmacokinetic properties of this complex were evaluated. Although HP- β -cyclodextrin consists of a mixture with a specific range of molecular weight, the risk of the product quality control and scale-up was effectively mitigated.

Results

Establishment of the standard curve of mebendazole

A solution of mebendazole in formic acid with a concentration of 1 mg/mL was prepared and used for UV spectrum scanning in the range of 200–400 nm; the maximum absorption of mebendazole at 234 nm was determined and used for HPLC analysis with a UV detector.

A series of solutions of mebendazole in a mixture of water and formic acid (10:1) with different concentrations were analyzed by HPLC with a UV detector at a wavelength of 234 nm. Based on the peak areas in the HPLC and the concentrations of mebendazole, it was found that, in the range of 0.01–0.1 mg/mL, the peak areas in HPLC were linearly related to the concentrations of mebendazole, and the linear regression equation was obtained as $Y = 13,547X - 24.476$ (determination coefficient R^2 : 0.9981, Y: absorption peak area, X: concentration). The standard curve is shown in Figure 2.

Phase solubility study

The solutions (10 mL) of β -cyclodextrin, γ -cyclodextrin, methyl- β -cyclodextrin, HP- β -cyclodextrin, and HP- γ -cyclodextrin in water with different concentrations were added to mebendazole and stirred at a speed of 200 r/min in a shaker at 25°C in the dark for 48 h. The selection of the concentrations of cyclodextrin derivatives for the solubility phase study was based on the solubilities of cyclodextrins. Each of the reaction mixtures (1 mL) was filtered and analyzed using HPLC. Based on the regression equation and standard curve, the amount of mebendazole in each solution was determined and summarized in Figure 3. The amounts of dissolved mebendazole in aqueous solutions of HP- β -cyclodextrin and methyl- β -cyclodextrin showed a linear increase in the range of 5 mmol/L to 25 mmol/L of the cyclodextrins, then plateaued and became independent of the cyclodextrin concentrations. The curves of HP- β -cyclodextrin and methyl- β -cyclodextrin can be considered as A_N -type diagrams. The cavity of HP- β -cyclodextrin exhibited better complex affinity than the others; the cavities of γ -cyclodextrin derivatives may be too large for mebendazole, β -cyclodextrin has the solubility issue, and HP- β -cyclodextrin may show better lipophilicity

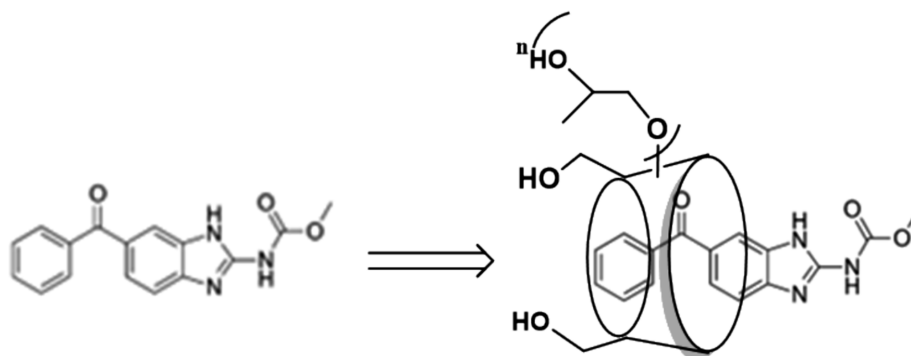


FIGURE 1
Structures of mebendazole and its complex with HP- β -cyclodextrin.

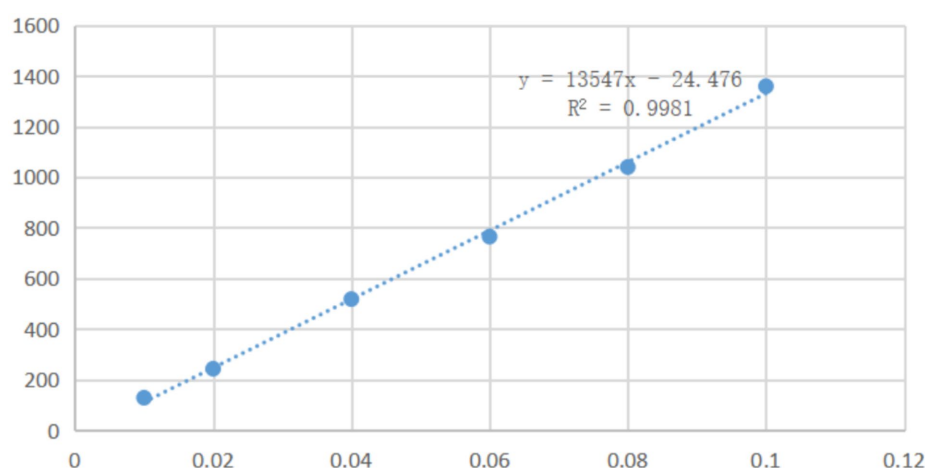


FIGURE 2
Standard curve and regression equation of mebendazole.

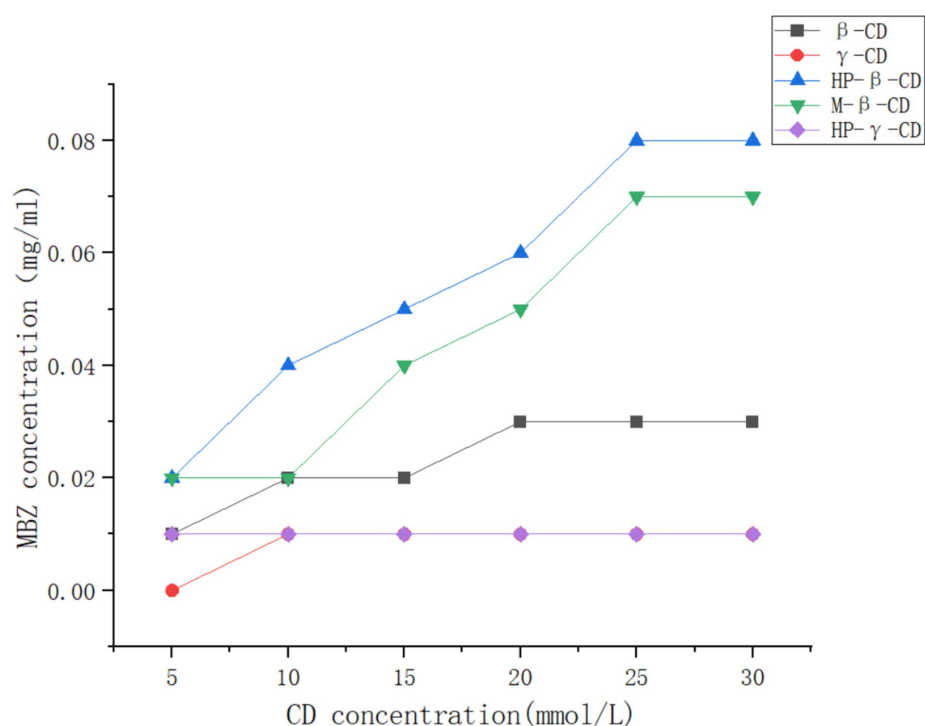


FIGURE 3
Solubilities of Mebendazole in the solutions of β-cyclodextrin, γ-cyclodextrin, methyl-β-cyclodextrin, HP-β-cyclodextrin, and HP-γ-cyclodextrin in water with different concentrations.

than methyl-beta-cyclodextrin. Therefore, HP-beta-cyclodextrin was selected to form a complex with mebendazole to prepare the complex.

Preparation of the complex of mebendazole with cyclodextrins

Cyclodextrin complex conditions, including reaction time, reaction temperature, stirring speed, and the ratio between mebendazole and HP-beta-cyclodextrin, could affect the inclusion results. Based on single factors and orthogonal strategies, many

complex conditions were tried, and water solubility was used as the criterion to judge the inclusion effect. The condition that was found to prepare the complex of mebendazole with HP-beta-cyclodextrin exhibited the best water solubility at 6.05 mg/mL so far, with 28% of inclusion rate and 90% of inclusion yield.

Characterization of mebendazole complex

The complex was confirmed by SEM in its solid state and by NMR spectra in its liquid state, and used to study the PK properties.

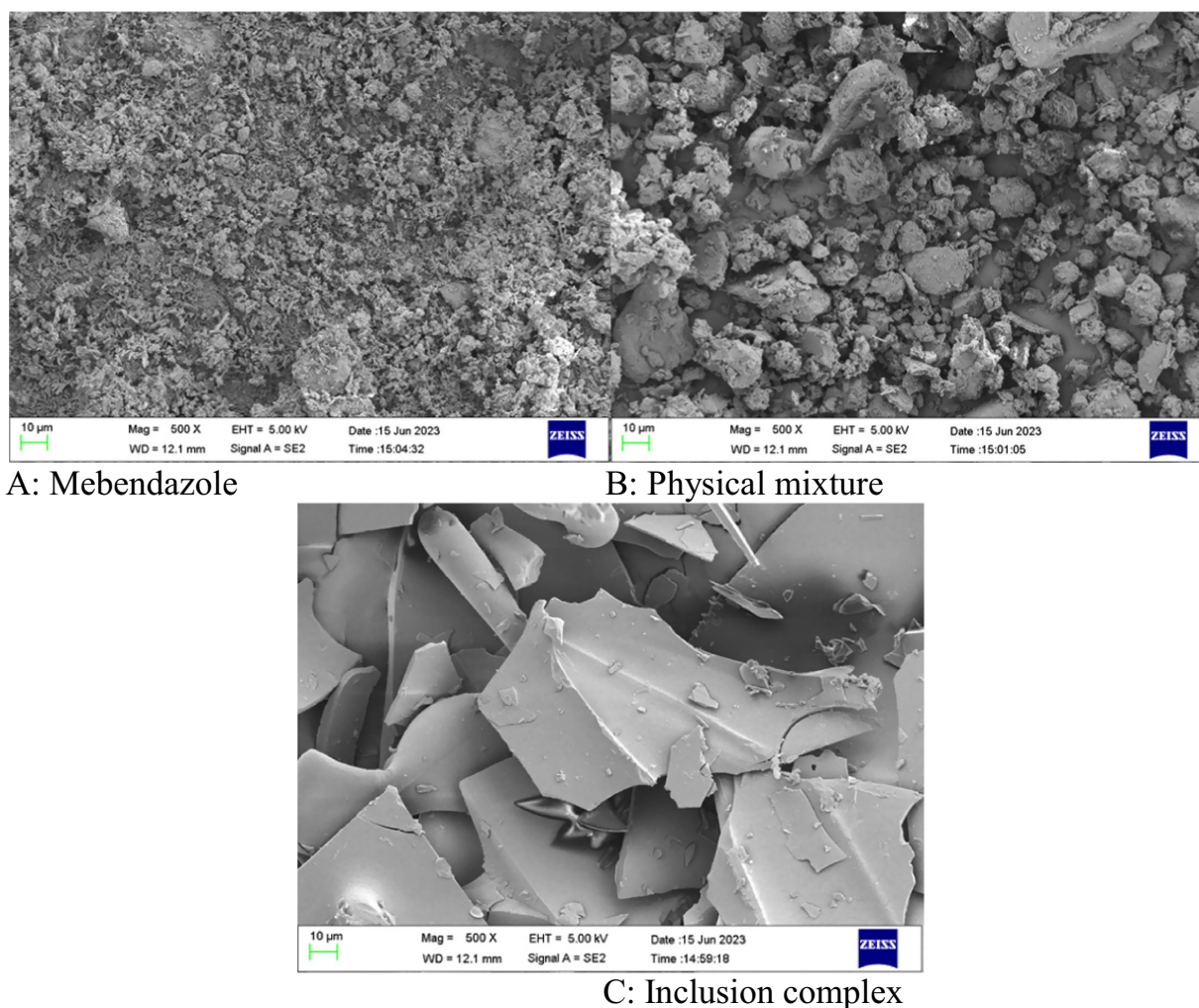


FIGURE 4

(A–C) The scanning electron microphotographs of mebendazole, the physical mixture of mebendazole and HP-beta-cyclodextrin, and the inclusion complex of mebendazole and HP-beta-cyclodextrin.

Mebendazole, the physical mixture of mebendazole and HP-beta-cyclodextrin, and their inclusion complex were checked by scanning electron microphotographs (Figure 4). Mebendazole shows fine crystals (A) that appear in the micrograph of the physical mixture (B), and is different from the microphotograph of the inclusion complex (C). The significant difference in the particle size and morphology of the inclusion complex from mebendazole crystals indicates the formation of a new phase.

NMR spectroscopy, as the most effective method, was used to confirm the formation of the mebendazole inclusion complex and the interaction between mebendazole and HP-beta-cyclodextrin in the complex. The 1D proton NMR and ROESY spectra of the inclusion complex in D₂O were recorded and are shown in Figure 5. The signals at 7.97 ppm (1H, s), 7.74 ppm (1H, d), 7.63 ppm (2H, m), 7.50 ppm (3H, m) and 7.40 ppm (1H, d) in the proton NMR spectrum of the inclusion complex in D₂O indicated that the complex contained the mebendazole molecules. The ROESY spectrum of the inclusion complex exhibited interactions between the protons at 7.97 ppm, 7.74 ppm, 7.63 ppm, 7.50 ppm, and 7.40 ppm from mebendazole and the protons at 3.80 ppm from

HP-beta-cyclodextrin, indicating that mebendazole was in the cavity of HP-beta-cyclodextrin.

The dissolution rate study

Mebendazole, the physical mixture of mebendazole and HP-beta-cyclodextrin, and the inclusion complex of mebendazole and HP-beta-cyclodextrin were used to study the dissolution rate, each experiment was repeated three times, with the standard deviations 0.01 to 0.09 for mebendazole, 0.15 to 0.42 for the complex, and 0.04 to 0.26 for the physical mixture, the results are shown in Figure 6.

In vivo pharmacokinetic study

In the range of 0.005 µg/mL to 80 µg/mL, the peak areas in HPLC and the concentrations of mebendazole in blank dog plasma showed the linear relationship, the dog plasma did not interfere the detection of mebendazole, and the standard curve and regression equation

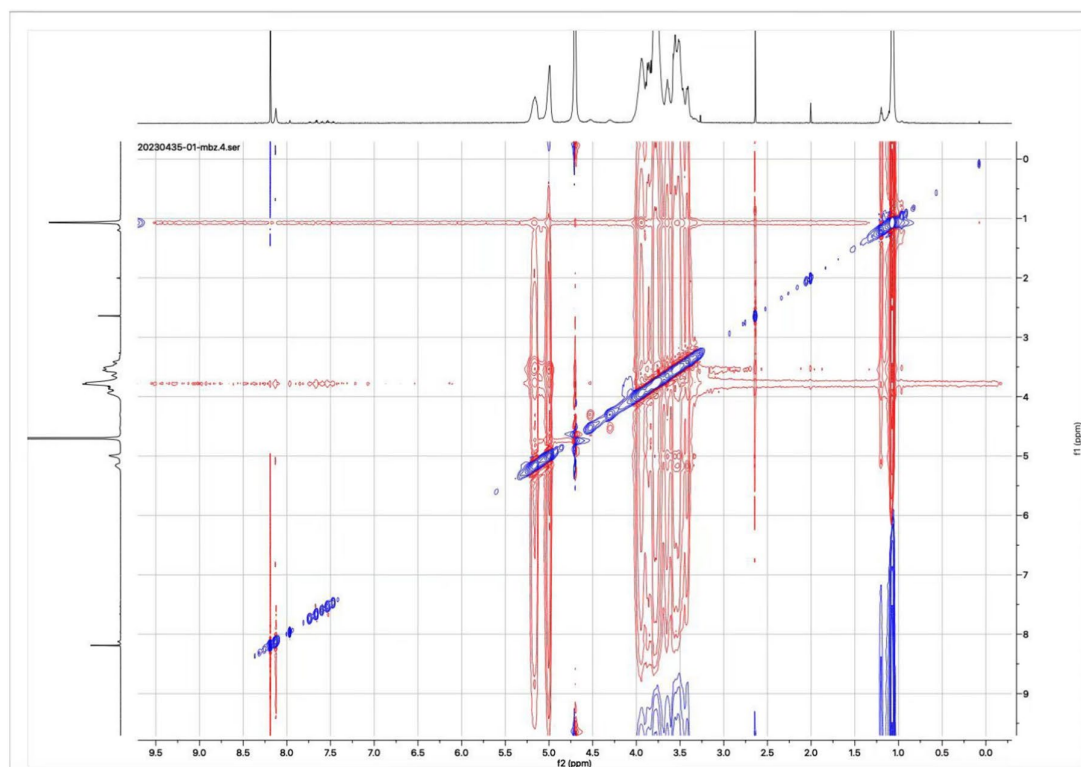
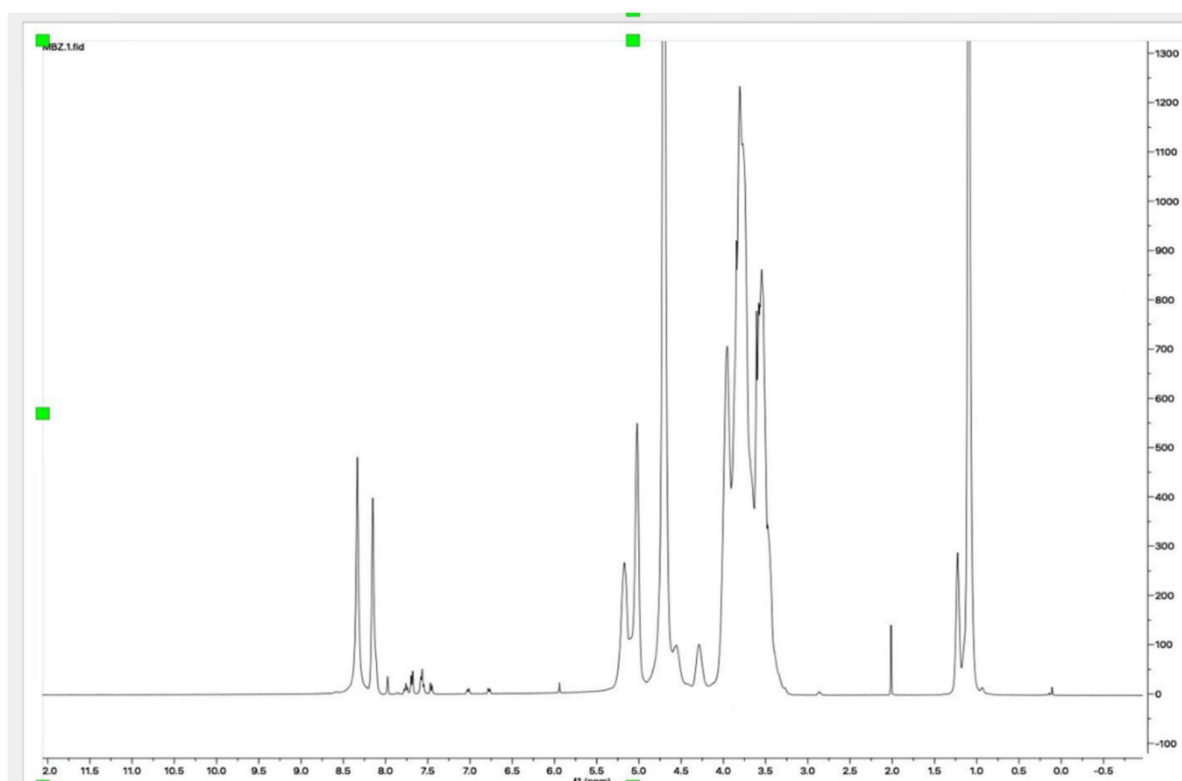


FIGURE 5

Proton NMR and ROESY spectra of the inclusion complex of Mebendazole with HP- β -cyclodextrin in D_2O .

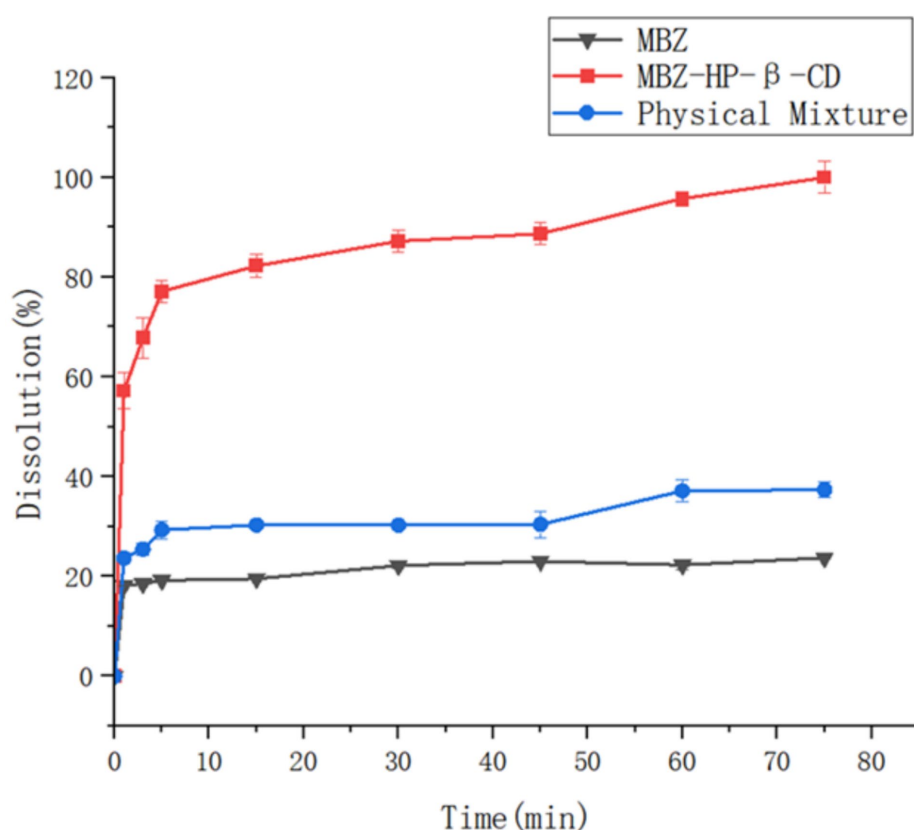


FIGURE 6

The dissolution rate curves of mebendazole, the physical mixture of Mebendazole and HP-beta-cyclodextrin, and their inclusion complex.

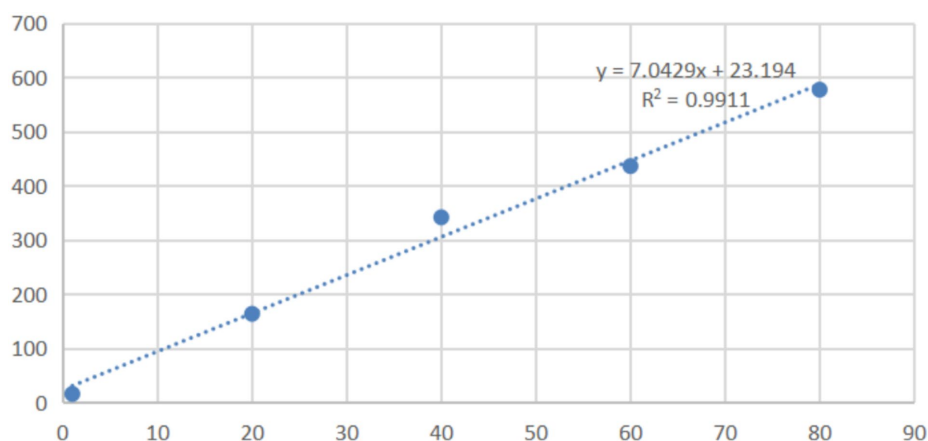


FIGURE 7

Standard curve and regression equation of mebendazole in blank dog plasma.

($Y = 7.0429X + 23.194$, $R^2 = 0.9911$) of mebendazole in dog plasma were obtained and shown in Figure 7 with LLOD of $0.005 \mu\text{g/mL}$ and LLOQ of $0.015 \mu\text{g/mL}$.

Twelve dogs ($5 \pm 0.1 \text{ kg}$), with half male and half female, were randomly divided into two groups as complex group and drug group, numbered and orally administered mebendazole or its inclusion complex at a dose of 5 mg/kg . The blood samples were collected by

using heparinized tubes, centrifuged, and kept in a -20°C freezer. The thawed plasma sample was extracted with organic solvents, centrifuged, filtered through a $0.22 \mu\text{m}$ microporous filter membrane, and analyzed by HPLC at room temperature.

The HPLC analysis of the plasma samples was conducted in a short time by using the same HPLC instrument and C-18 column; each time, before and after the HPLC analysis, the standard sample of

mebendazole was checked for calibration to avoid system errors. The plasma samples were analyzed by HPLC.

Based on the obtained HPLC data, the standard curve of mebendazole in plasma and the regression equation, the relationship between the concentration of mebendazole in plasma from dogs dosed with mebendazole or its inclusion complex and the collection time was obtained to produce the drug-time curve shown in Figure 8. The PK parameters were obtained by processing the data using WinNolin software, and the main pharmacokinetic parameters are summarized in Table 1.

Discussion

Many methods can be used to confirm the formation of inclusion complexes of mebendazole with cyclodextrin derivatives, such as FTIR, DSC, TAG, X-ray, SEM, and mass spectra; however, these

methods are not accurate and have limited correlation with mebendazole’s water solubility. In the majority of cases, the main purpose of preparing the inclusion complex of mebendazole is to increase its water solubility. NMR spectra is the most powerful tool that not only confirms the interactions between mebendazole and cyclodextrin derivatives in the complex but also indicates the water solubility of mebendazole in the complex when D₂O was used as the solvent.

In 2007, mebendazole was complexed with different cyclodextrins, including α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, HP-α-cyclodextrin, HP-β-cyclodextrin, HP-γ-cyclodextrin, and permethyl beta-cyclodextrin in a mixture of acetone or acetonitrile and water, with or without formic acid. Permethyl beta-cyclodextrin could increase the water solubility of mebendazole by 4,700 times (34). The same result was reported in 2008 (36), and the compound permethyl beta-cyclodextrin was defined as partially methylated beta-cyclodextrin. The mass spectrum of the complex was recorded, but the

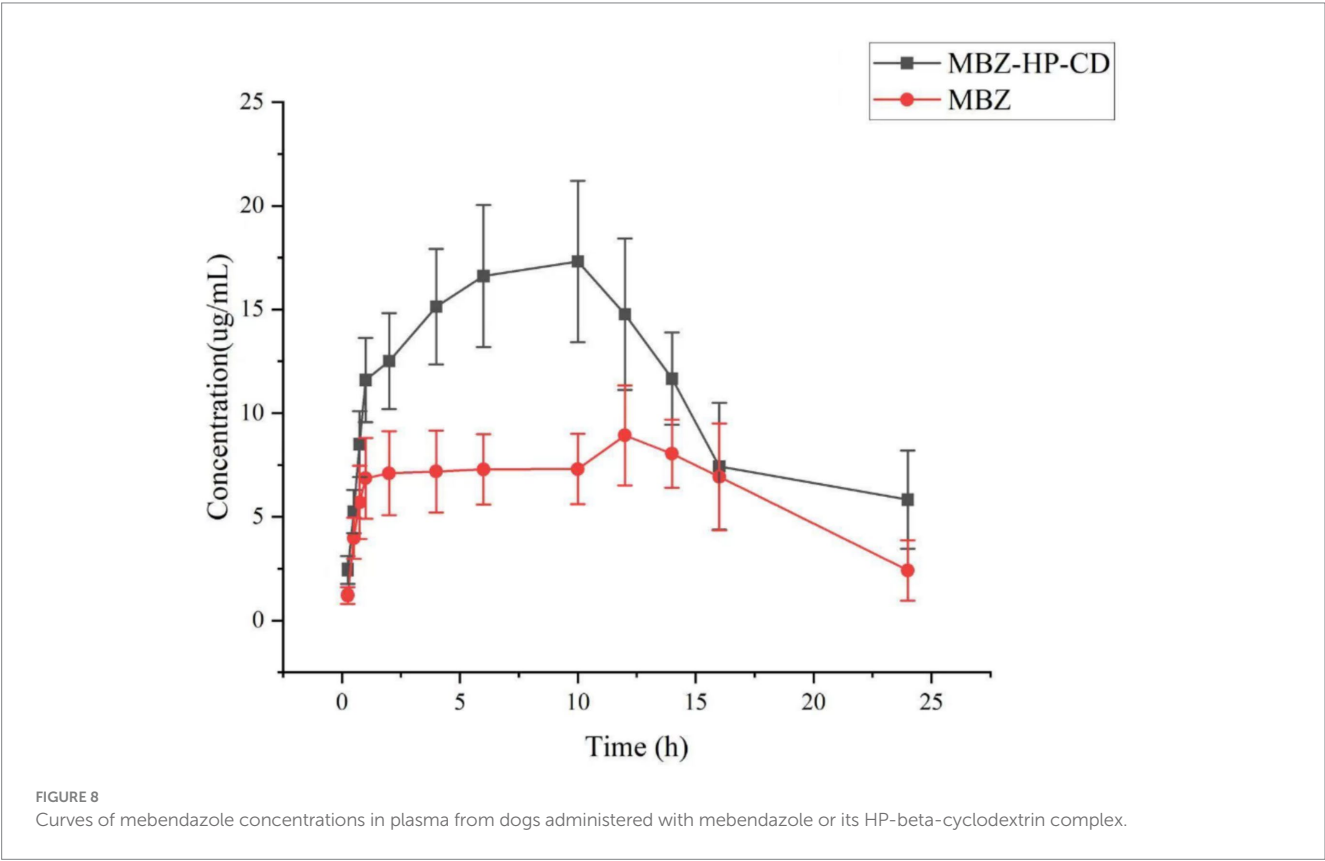


TABLE 1 Pharmacokinetic parameters of icariin and its HP-beta-cyclodextrin complex.

| Parameters | Unit | Value | |
|---------------------|---------|---------------|----------------------------------|
| | | Mebendazole | Mebendazole/HP-beta-cyclodextrin |
| T _{max} | H | 12.00 ± 0.50 | 10 ± 0.50 |
| C _{max} | µg/mL | 8.96 ± 0.15 | 17.34 ± 2.02 |
| AUC ₀₋₂₄ | µg·h/mL | 151.32 ± 5.92 | 289.02 ± 15.83 |
| t _{1/2} | H | 5.81 ± 0.36 | 10.01 ± 2.07 |
| MRT | H | 13.35 ± 0.30 | 16.85 ± 0.22 |

mass peak for the complex (mebendazole + permethyl beta-cyclodextrin) was not detected; the NMR spectrum of the complex in D₂O was not recorded. In the present study, the complex of mebendazole with methyl beta-cyclodextrin was confirmed by proton NMR and ROESY spectra in D₂O.

In 1999, the NOESY spectrum of mebendazole and γ -cyclodextrin or α -cyclodextrin was recorded (30), and the NOESY signals indicated the interaction between the mebendazole and cyclodextrins. However, the NMR spectrum was recorded in DMSO-d₆, which indicated that the complex was formed in DMSO and had less relation to the drug's water solubility. The proton NMR and ROESY spectra in D₂O can not only confirm the formation of the complex, but also indirectly confirm the complex's water solubility.

The inclusion complex of mebendazole and *citrate*-beta-cyclodextrin was prepared in the mixture of formic acid and water, but the water solubility of mebendazole was increased only four-fold (33). As mentioned in the introduction, the solubility increase of cyclodextrin complexations in other literature reports is in the range of 4-fold to 2020-fold, and the complexes and solubility were not confirmed by NMR spectra in D₂O.

In summary, the formulation in this study showed the best mebendazole water solubility increasing so far. The solubility and interaction between mebendazole and HP-beta-cyclodextrin in the complex were confirmed by proton NMR and ROESY spectra in D₂O.

The dissolution rate studies showed that 80% of mebendazole was released from the inclusion complex within 15 min, whereas under the same conditions, only 30% of mebendazole was released from its physical mixture, and only 20% was released from the pure mebendazole.

The *in vivo* PK study showed that through complexation with HP- β -cyclodextrin, C_{\max} of mebendazole was increased from $8.96 \pm 0.15 \mu\text{g/mL}$ to $17.34 \pm 2.02 \mu\text{g/mL}$, T_{\max} of mebendazole was shortened from $12.00 \pm 0.50 \text{ h}$ to $10 \pm 0.50 \text{ h}$, the half life time was prolonged from $5.81 \pm 0.36 \text{ h}$ to $10.01 \pm 0.07 \text{ h}$, the AUC₀₋₂₄ was increased from $151.32 \pm 5.92 \mu\text{g}\cdot\text{h/mL}$ to $289.02 \pm 15.83 \mu\text{g}\cdot\text{h/mL}$, and the bioavailability was increased 91%.

Oral medications are typically swallowed and absorbed in the stomach or small intestine before entering the portal venous system and passing through the liver, and then into the systemic circulation.

When the drug is taken orally, it is broken down by stomach acids before it passes through the liver and then enters the bloodstream. When the medication is coated or complexed with cyclodextrin derivatives, the drug is transferred into the stomach much more than the drug alone. It is not surprising that the C_{\max} of mebendazole in the complex group is larger than the C_{\max} in the pure drug group. The solubility of mebendazole in complex is better than the pure drug, and it will take a longer time for the pure drug to dissolve. Therefore, T_{\max} in the complex group will be shorter than that of the pure drug group. Since the blood drug concentration in the complex group was higher than that in the pure drug group, it is no wonder that the half-life and MRT of the drug in the complex group were longer than those in the pure drug group.

Materials and experiments

Methyl β -cyclodextrin (DS: 11–13), HP β -cyclodextrin (average Mw: 1460), HP γ -cyclodextrin (DS: 4–6), β -cyclodextrin, and γ -cyclodextrin were purchased from Shanghai McLean Biochemical

Technology Co., Ltd. (Shanghai, P. R. China). Mebendazole ($\geq 99\%$) was purchased from Beijing Solebao Technology Co., Ltd. (Beijing, P. R. China). Formic acid, acetonitrile, and methanol were obtained from Xilong Science Co., Ltd. (Guangzhou, P. R. China). The dogs were purchased from Guangdong Medical Laboratory Animal Center.

Preparation of the solution of mebendazole

Mebendazole (10 mg) in a 10 mL volumetric flask had a small amount of formic acid (1 mL) added, then it was vortexed and pipetted with water (9 mL) to provide a solution with a concentration of 1 mg/mL.

UV spectral screening of the standard solution of mebendazole

The standard mebendazole solution (1 mg/mL) was vortexed, filtered through a membrane into a cuvette, and scanned using a UV spectrophotometer (from Shanghai Jinghua Technology Instrument Co., Ltd.) in the wavelength range of 200–400 nm at room temperature.

HPLC standard curve and regression equation establishment

A solution of mebendazole (1 mg) in formic acid (1 mL) was diluted with water to obtain a series of samples with concentrations of 0.01 mg/mL, 0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL and 0.1 mg/mL for HPLC analysis. An LC-15C high-performance liquid chromatograph (from Shimadzu Enterprise Management, China Co., Ltd.) was used to analyze the samples, with a UV detector at 234 nm on a Shimpack VP ODS C18 column (250 \times 4.6 mm). The mobile phase was acetonitrile and water (50:50), the flow rate was 1.0 mL/min, and the testing temperature was 30°C. Based on the peak areas in HPLC and the concentrations of mebendazole in solutions, the standard curve (shown in Figure 2) and a linear regression equation were obtained.

Phase solution study

The solutions of β -cyclodextrin, γ -cyclodextrin, methyl- β -cyclodextrin, HP- β -cyclodextrin, and HP- γ -cyclodextrin in ultrapure water (10 mL) with concentrations of 5 mmol/L, 10 mmol/L, 15 mmol/L, 20 mmol/L, 25 mmol/L, and 30 mmol/L had extra mebendazole added in a shaker at 25°C in the dark, and were shaken for 48 h. After filtration, 1 mL of each reaction was taken for HPLC analysis to provide the phase solution study results (shown in Figure 3).

Water solubility determination of mebendazole in complex

An extra mebendazole complex was added to water (1 mL) while shaking. After 2 h, the mixture was filtered and analyzed by HPLC to obtain the water solubility of mebendazole in the complex.

Determination of the inclusion rate and inclusion yield

The formulas shown below were used to calculate the inclusion rate and yield of mebendazole in complexes:

$$\text{Inclusion yield (\%)} = \left[\frac{\text{complex (mg)} / \text{mebendazole (mg)}}{+\text{cyclodextrin (mg)}} \right] \times 100\%.$$

$$\text{Inclusion ratio (\%)} = \left[\frac{\text{mebendazole in complex (mg)}}{/\text{mebendazole (mg)}} \right] \times 100\%.$$

Preparation of the complex of mebendazole with HP-beta-cyclodextrin

HP-beta-cyclodextrin (365.4 mg, 0.25 mmol) was slowly added to the solution of mebendazole (74 mg, 0.25 mmol) in formic acid (8 mL), stirred at 500 r/min at 50°C for 3 h, then cooled in a refrigerator at 4°C for 8 h, evaporated to remove the formic acid (until no formic acid smell), dissolved into water (50 mL), filtered through a 0.22 µm filter membrane, cooled at -20°C overnight, and freeze-dried under reduced pressure to obtain the inclusion complex as a solid powder.

Preparation of the physical mixture of mebendazole and HP-beta-cyclodextrin

The grounded mebendazole (74 mg, 0.25 mmol) was mixed with grounded HP-beta-cyclodextrin (365.4 mg, 0.25 mmol) and passed through an 80-mesh sieve to provide the physical mixture of mebendazole and HP-beta-cyclodextrin.

Scanning electron microscopy

Mebendazole, the physical mixture of HP-beta-cyclodextrin and mebendazole, and the inclusion complex were gold-plated, placed on the sample stage, and scanned with a scanning electron microscope (Merlin from the German Zeiss company) with 5 kV accelerating voltage.

NMR study

The proton NMR spectrum of mebendazole complex with HP-beta-cyclodextrin in D₂O was recorded using a Bruker 400 NMR spectrometer (from Germany), and the ROESY spectrum was recorded using a Bruker 600 NMR spectrometer (from Germany).

The dissolution rate study

Based on the paddle method in the first part of the 2010 edition of the Chinese Veterinary Pharmacopeia, the release cups containing

ultrapure water (900 mL) degassed by an ultrasonic cleaning machine were placed with mebendazole (50 mg), the complex of mebendazole and HP-β-cyclodextrin (contained 50 mg of mebendazole), and the physical mixture of mebendazole and HP-beta-cyclodextrin (containing 50 mg of mebendazole), and stirred at speed of 100 r/min at 37 ± 0.5°C, respectively. Samples (1 mL) were taken at 1, 3, 5, 15, 30, 45, 60, and 75 min from each cup and filtered through a 0.22 µm microporous filter membrane within 30 s for HPLC analysis, at the same time, 1 mL of the isothermal dissolution medium was added to the cup. Each experiment was repeated three times, and based on the HPLC results, the time-cumulative dissolution curve was obtained and shown in [Figure 6](#).

In vivo PK study

Establishment of the standard curve of mebendazole in blank dog plasma

Mebendazole (10 mg) was dissolved into chromatographic pure acetonitrile (2 mL), diluted with chromatographic pure methanol, and added to tubes containing 0.2 mL of blank dog plasma (received from Guangzhou Rui-Te Co., Ltd., Guangzhou, Guangdong, China) to obtain the solutions with concentrations of mebendazole as 0.015 µg/mL, 20 µg/mL, 40 µg/mL, 60 µg/mL, and 80 µg/mL for HPLC analysis. The mobile phase was a gradient of 0.3% potassium phosphate solution and acetonitrile, and the pH of the mobile phase was adjusted to 4 by adding triethylamine. The wavelength of the UV detector was 234 nm, the column temperature was 35°C, the flow rate was 1 mL/min, the injection volume was 50 µL, and gradient elution was as follows: 0–14 min, 25% acetonitrile, 14–18 min, 40% acetonitrile, 18–25 min, 25% acetonitrile. Under these HPLC conditions, the dog plasma did not interfere with the detection of mebendazole. Based on the HPLC data, the standard working curve of mebendazole in blank dog plasma and the regression equation were obtained, as shown in [Figure 7](#).

Recovery rate of mebendazole from the dog plasma and intra-day and inter-day precision and accuracy

An appropriate volume of the mebendazole standard solution was added to 500 µL of dog blank plasma to prepare the samples with different concentrations of mebendazole: 10 µg/mL, 1 µg/mL and 0.1 µg/mL. After extraction and filtration, the samples were analyzed by HPLC. Five parallel trials were performed at each concentration, three samples of each concentration were prepared, the intra-day sample was measured three times, and the intra-day difference coefficient was calculated. The test was carried out continuously for 3 days, and the coefficient of inter-day variance was calculated. The recovery rates were obtained in the range of 110% ± 0.25 to 90.8% ± 0.27%. The intra-day difference coefficient was 1.14% ~ 5.67%, and the inter-day difference coefficient was 1.4% ~ 3.5%.

Blood sample collection

A total of 12 healthy adult dogs (5 ± 0.1 kg). The group consisted of an equal number of male and female dogs (sourced from Guangdong Medical Laboratory Animal Center, Guangzhou, Guangdong, China). We informed the center that these dogs would be used for an *in vivo* PK study. The dogs were randomly divided into two groups, named as

mebendazole group and the inclusion group. They were weighed, numbered, and fed a diet of low-fat dog food (obtained from Shanghai Jibai Chong Industrial Co., Ltd., Shanghai, China) for 1 week in a warm and ventilated environment. Prior to administration, the dogs were fasted for 12 h. In addition, they were dosed orally with mebendazole or its complex at a dosage of 5 mg/kg, and they had access to normal drinking water during sampling. Blood samples (2 mL) were taken from the forearm vein at 0.25, 0.5, 0.75, 1, 2, 4, 6, 10, 12, and 24 h after administration. The samples were immediately transferred to heparinized tubes, centrifuged at 3500 rpm for 10 min, and stored in a freezer at -20°C .

Analysis of the blood samples

The plasma sample was thawed at room temperature, and 0.5 mL was pipetted into a 2 mL centrifuge tube. A mixture of dichloromethane and methanol (1:1, 1 mL) was added, and the solution was vortexed for 2 min. It was then centrifuged at a speed of 13,000 r/min for 10 min and transferred to a test tube. The procedure was repeated twice. The resulting solution was evaporated to dryness under a nitrogen stream at 40°C . Following this, 0.5 mL of acetonitrile was added, and the mixture was vortexed for 5 min. It was then centrifuged at 13000 r/min for 10 min, and filtered through a $0.22\text{ }\mu\text{m}$ microporous filter to prepare the sample for HPLC analysis.

Statistical analysis

All analyses were carried out in triplicate, and statistical analysis was performed using one-way analysis of variance, followed by a significant difference test using Prism 9 software. $p \leq 0.05$ indicates that the difference is statistically significant.

Conclusion

Using cyclodextrin and complexation condition selections based on single factors and orthogonal strategies, an inclusion complex of mebendazole/HP-beta-cyclodextrin with a water solubility of 6.05 mg/mL was prepared and confirmed by SEM and NMR spectra. In dissolution rate studies, 80% of mebendazole was released from the complex in 5 min. In an *in vivo* PK study, the bioavailability increased by 91%, indicating that the prepared mebendazole/HP-beta-cyclodextrin complex could be used as a potential anti-cancer agent in clinical practice. Additionally, *in vivo* efficacy study, toxicity study, and immunogenicity assessment are in progress.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

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Ethics statement

All methods in the study were performed in accordance with the relevant guidelines and regulations in ARRIVE guidelines. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

CD: Investigation, Writing – original draft, Data curation. YD: Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. ZX: Investigation, Methodology, Writing – original draft. PW: Investigation, Methodology, Writing – original draft. SX: Investigation, Methodology, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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