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Original Article

Construction of Metronidazole Capped in Gold Nanoparticles against *Helicobacter pylori*: Antimicrobial Activity Improvement

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Abstract

Introduction: *Helicobacter pylori* is considered a major agent causing gastritis and peptic ulcer disease. Unfortunately, the occurrence of increasing drug resistance to this bacterium would result in some difficulties in its treatment. Therefore, the application of nanotechnology has been suggested to resolve such problems. Nanoparticles usage in medical research has been expanded in recent years. Among nanometals, gold nanoparticles have exclusive features that can be used in such applications. Using nanotechnology in medical science could help mankind to solve this problem in the future.

Aim: Our aim in this research was to investigate the antimicrobial effect of gold nanoparticles on *H. pylori* strains.

Materials and methods: Gold nanoparticles were synthesized by the Turkevich method. Then, their size and dispersion were investigated using spectrophotometry, DLS, and TEM microscopy. Subsequently, the combination of metronidazole and gold nanoparticles was obtained by mixing method, and then the anti-helicobacter effects of the two were evaluated according to CLSI.

Results: The highest size of gold nanoparticles was between 12 and 9 nm, and the maximum absorbance was 522 nm; however, in conjugated state, the maximum absorbance was 540 nm, which indicated the accumulation of drug-conjugated nanoparticles in the conjugate state. Some changes indicated the binding of metronidazole to gold nanoparticles. Antimicrobial testing of gold nanoparticles and metronidazole did not affect the *Helicobacter pylori*. Therefore, the combination of gold nanoparticles and metronidazole had a 17-mm growth inhibition zone.

Conclusions: The anti-helicobacter effects of metronidazole significantly increased in conjugation with gold nanoparticles.

Keywords

antimicrobial activity, capped, *Helicobacter pylori*, gold nanoparticles, metronidazole

INTRODUCTION

Helicobacter pylori is a helical shape bacterium in the mucous membrane layer of the human stomach that is associated with digestive diseases.[1] This bacterium is considered as an important factor in causing chronic gastritis and also in gastric ulcer and gastric cancer.[2,3] In addition, *H. pylori* is associated with the Mucosa Associated Lymphoid Tissue (MALT) and also correlations with the extra-digestive diseases were reported.[4,5] It is estimated that more than half of the world's population is infected with *H. pylori*. It is now recognized that the emergence of antibiotic resistance is the main obstacle in the eradication of *Helicobacter pylori*.[6] Infection rates differ between the developed and developing countries worldwide. Because of the combination therapies, better personal hygiene and community health, *Helicobacter pylori* infection is severely reduced in most Western countries.[7] Resistance to metronidazole is highly variable among the *H. pylori* strains.[8] Metronidazole resistance is even more prevalent (up to 95% of isolates) in developing countries.[9] The emergence of resistant strains to current antibiotics has become a serious problem for public health.[10] Therefore, development of new bactericides seems to be necessary. The term "Nanomedicine" is used for applying nanotechnology in diagnosis, treatment, monitoring, and control of diseases.[11] Nanotechnology is rapidly evolving from new concepts of medicine and drug delivery and diagnostic methods in all fields of medicine.[12] Nanoparticles appear to have a wide range of uses due to their unique properties, drug delivery and drug release. Among them, gold nanoparticles already have good properties for binding to drugs and delivering them to the target site.[13,14] The chemical, optical and electronic properties of gold nanoparticles make these materials well used in drug delivery.[15] The increased drug concentration at infection site and reduction of the toxicity of the drug were reported as the main advantages of gold nanoparticles as drug carriers.[16] Due to the drug resistance in bacteria and the lack of recent antibiotic detection, optimizing the optimal delivery of the drug to the target site in the bacterium will defeat the resistance of the pathogenic bacterium. Recently, studies have been conducted on the use of antibiotic-coated gold nanoparticles, so no studies have been performed on the *Helicobacter pylori*. In the present study, a simple method for binding metronidazole to gold nanoparticles is proposed. Various analytical techniques such as UV-vis spectrum, FT-IR, and electron microscopy analysis were used to investigate the interaction between metronidazole and gold nanoparticles. In addition, the antibacterial effect of drugs coated with gold nanoparticles against metronidazole-resistant *H. pylori* strain was investigated.

AIM

The aim of this study was to investigate the impact of gold nanoparticles conjugated with metronidazole against metronidazole-resistant *H. pylori* isolate.

MATERIALS AND METHODS

$HAuCl_4 \cdot 3H_2O$ and metronidazole were purchased from Alfa Asear (USA), and Sigma (USA), respectively. Trisodium citrate, potassium bromide (spectroscopic grade), Brucella agar and Muller-Hinton agar were obtained from Merck company (Germany).

Methods

Preparation of gold nanoparticles

Trisodium citrate (1 M, 0.5 mL) and double distilled water (18.5 mL) were added to the boiling solution of $HAuCl_4 \cdot 6H_2O$ (1 mM, 5 mL), to obtain a wine red solution from a previously yellow one and then the gold nanoparticles were shown by the absorption at ultraviolet spectrum, and the shape of the gold nanoparticles was measured using TEM and particle size distribution by DLS.[17]

Preparation of metronidazole coated gold nanoparticles

The drug-coated nanoparticles were prepared as follows: 0.1 mM citrate stabilized gold nanoparticles [10 mL of 0.5 mM Au (0) was diluted 1:4 in water and mixed with 5 mL of 3 mM metronidazole. Then they were effectively stirred for 2 h.[17]

Device analysis

UV–vis spectrum was measured using a Carry 100 spectrophotometer. The DLS was used for analysis of Zeta potential and gold nanoparticle's size. The free state of nanoparticles and its conjugate with metronidazole was observed in the transmitting electron microscope, with an acceleration potential of 120 KV. The infrared spectrum was used to investigate changes in the metronidazole functional group.

Anti-Helicobacter assay

Helicobacter pylori from the gastric sample of patients with clinical symptoms referred to the endoscopy department of the hospital in Tehran was obtained. Biopsy samples after homogenization were then cultivated in 10% sheep's blood agar Brucella culture medium containing three antibiotics: vancomycin (10 mg/L), amphotericin B (10 mg/L), and trimethoprim (5 mg/L). Then they were incubated. The petri dishes were incubated at 37°C under microaerophilic atmosphere prepared by Anerocult C (Merck, Germany) for 3-5 days. The isolates were tested by hot staining and urease, catalase, oxidase test and identified as *H. pylori*.[1] The CLSI recommended method called modified disc diffusion method was used for drug susceptibility testing. In summary, a 4 McFarland turbidity (12×10^8 CFU/ml) of bacterial suspensions was prepared and cultivated in 10% defibrinated sheep blood supplemented Muller-Hinton agar. The 5 µg metronidazole discs (Mast, England) were placed in the plates and then incubated to

microaerophilic atmosphere for 3 days at 37°C. The inhibition zone of ≥ 21 mm was considered susceptible, and the less were considered resistant.[1] Then, metronidazole and gold nanoparticle-coated metronidazole discs were placed on blood MHA agar plates. Since then, the plates have remained at a temperature of 25°C for 1 hour to minimize pre-incubation diffusion to minimize the effects of time changes between applications of different solutions. The inoculated plates were incubated at 37°C for 3 to 5 days in the microaerophilic atmosphere. Finally, they were observed for antibacterial activity by determining the diameter of the inhibitory zone.

RESULTS

UV spectrum, DLS and TEM assays

The characteristic of gold nanoparticles was observed at a maximum wavelength for the red gold nanoparticles at 522 nm in the UV spectrum. Metronidazole was absorbed approximately at 340 nm. By adding gold nanoparticles, a new peak emerged at 540 nm **(Fig. 1)**. The maximum size frequency of gold nanoparticles was about 10 nm, and the zeta potential was reported by the Zeta analyzer about -27.5 **(Figs 2,3)**. The shape of the gold nanoparticles can be clearly seen in the TEM images **(Fig. 4a)**. TEM image of conjugated state, accumulation of nanoparticles has also been observed **(Fig. 4b)**.

FT-IR studies of drugs coated gold nanoparticles

The IR spectrum of metronidazole alone and combination with gold nanoparticles is shown in **Fig. 5**. The changes were observed in the conjugated spectrum rather than the state of free metronidazole within FT-IR analysis, which metronidazole show bands were near 2500-3000 cm^{-1}.

Microbial efficacies of GNP – metronidazole complex

Antibacterial efficacy of gold nanoparticles, metronidazole and gold nanoparticle-coated metronidazole against metronidazole-resistant *H. pylori* is shown in **Table 1**. The antibacterial activity of gold nanoparticle-coated metroni-

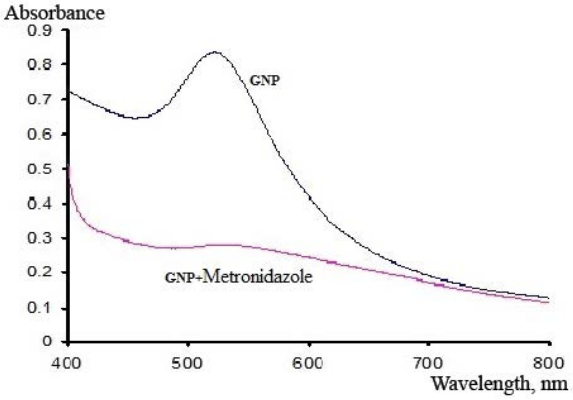

Figure 1. UV spectrum of gold nanoparticles after conjugation with metronidazole.

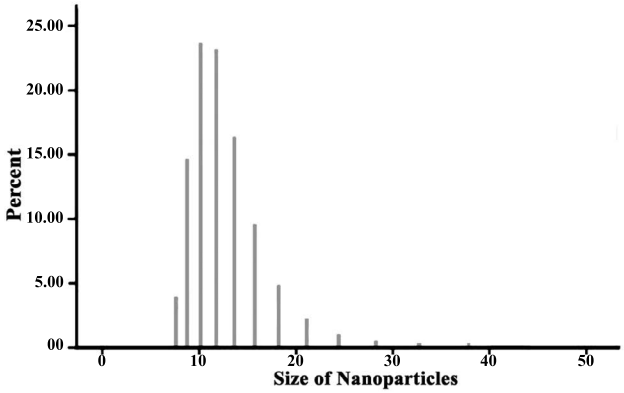

Figure 2. Scattering of gold nanoparticles.

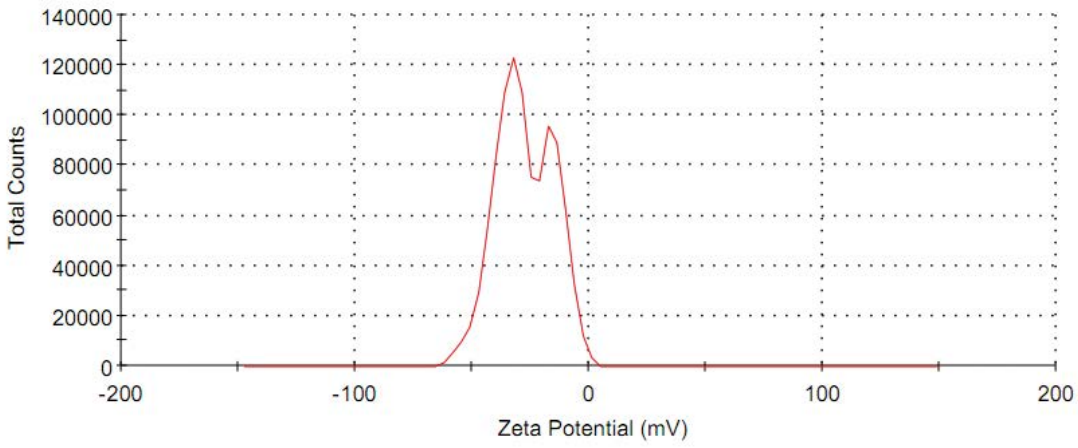

Figure 3. Zeta potential of gold nanoparticles.

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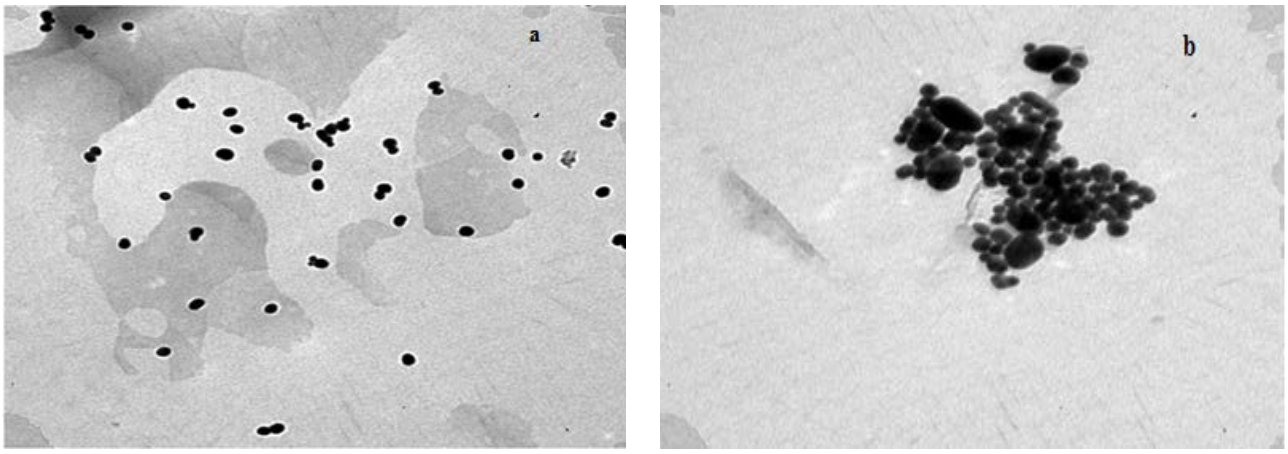

Figure 4. TEM of gold nanoparticles (a) and conjugated state (b).

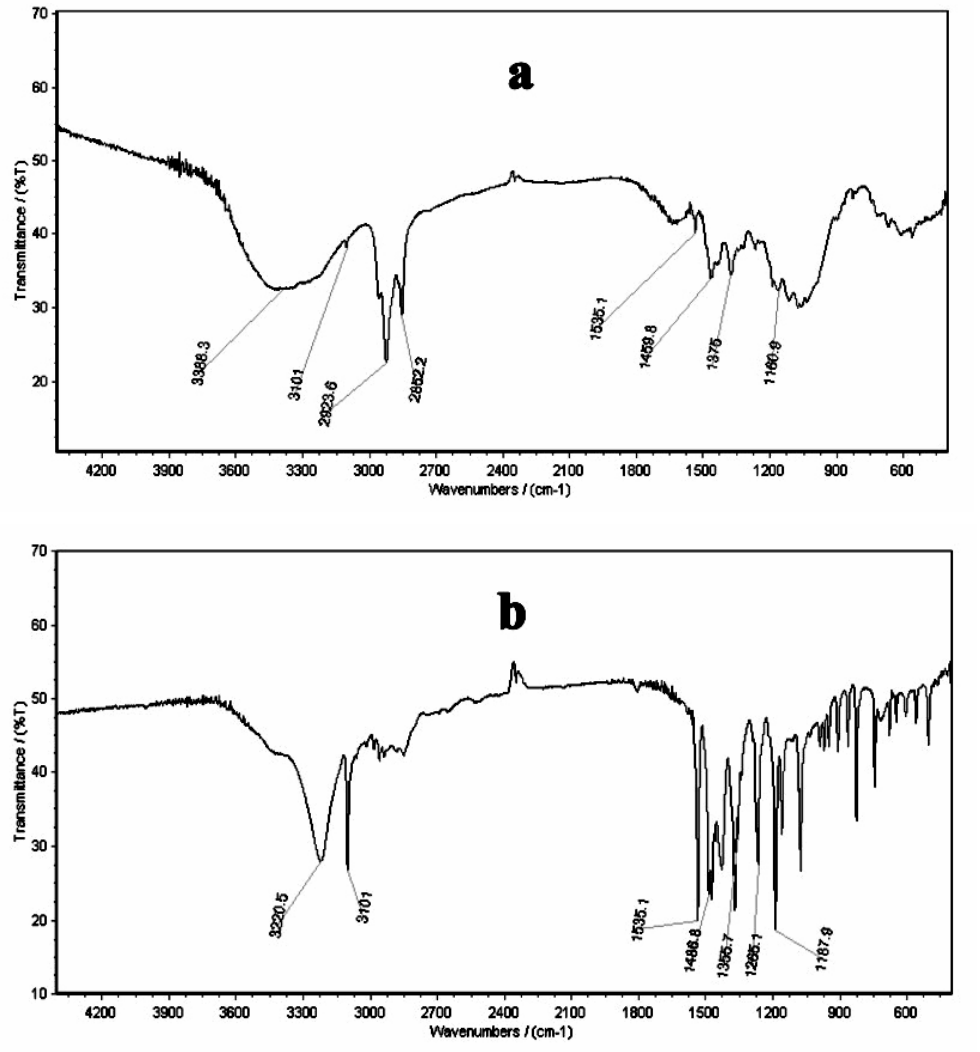

Figure 5. FT-IR spectrum of conjugated (a) and pure metronidazole (b).

Table 1. Anti-Helicobacter activity of gold nanoparticle and coated metronidazole

	gold nanoparticle	metronidazole	metronidazole – gold
Metronidazole resistant H. pylori	0 mm	0 mm	17 mm

dazole was higher than the pure metronidazole. The image of petri dish showing the microbial studies of pure metronidazole and gold nanoparticle-coated metronidazole is shown in **Fig. 6**.

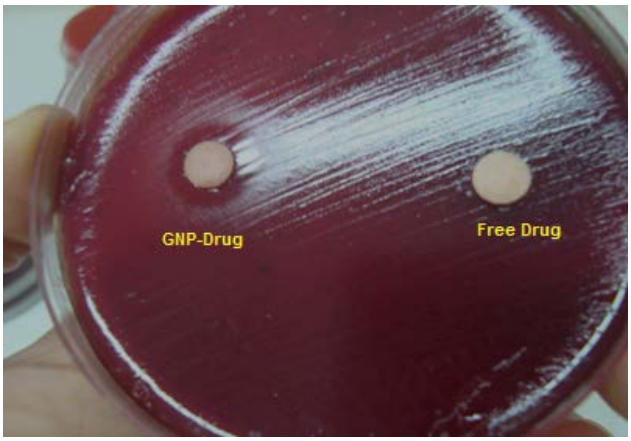

Figure 6. Antibacterial disk diffusion test.

DISCUSSION

Gold nanoparticles have no microbial activity and acts only as a carrier for drugs. The large surface area of Au (0) allows it to adsorb more drug molecules and carry a large number of drugs. According to the results, it has been verified that metronidazole-capped gold nanoparticles are effective against metronidazole-resistant *H. pylori* strain compared to the pure metronidazole. These gold particles surrounded by some drug moieties now act as a single group against the microbial organisms. During the metronidazole-gold nanoparticle conjugation, aggregation of the particles occurred, which consisted of gold atoms surrounded by some drug molecules. This made an effective approach to better combat the bacterial pathogens using the drug molecules as a group rather than acting alone, as evidenced by TEM analysis. Based on the studies, it can be verified that gold nanoparticles can act as an effective carrier or anchor for drugs. Grace et al.[17] reported that gold nanoparticles had no antibacterial effects against some microorganisms including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*, while coating the antibiotics with gold nanoparticles increased their antibacterial activity. However, recent studies by Burygin et al.[18] revealed that gold nanoparticles have no enhancing effect on the antibacterial activity of gentamycin when used separately or as a mixture.

Conclusions

Therefore, as suggested, it appeared that gold nanoparticles could enhance the antibacterial activity of antibiotics when antibiotics are chemically attached to the surface of gold nanoparticles. Hence, the stable form of antibiotic-gold nanoparticle conjugates is more effective rather than when they were used in combination with antibiotics as a mixture.

Conflict of Interest

None declared.

Author contribution

All authors were involved in the study design, data collection, concept, definition of intellectual content, literature search, experimental studies, data acquisition, data analysis, and manuscript preparation.

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Разработка метронидазола, покрытого золотыми наночастицами, против *Helicobacter pylori*: улучшение антимикробной активности

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Резюме

Введение: *Helicobacter pylori* считается основным возбудителем гастрита и язвенной болезни. К сожалению, растущая лекарственная устойчивость этой бактерии может привести к трудностям в её лечении, поэтому для решения таких проблем предлагается применение нанотехнологий. Использование наночастиц в медицинских исследованиях в последние годы расширилось. Среди нанометаллов наночастицы золота обладают исключительными характеристиками, которые можно использовать для таких приложений. Использование нанотехнологий в медицине может помочь человечеству решить эту проблему в будущем. Нашей целью в этом исследовании было изучить антимикробный эффект наночастиц на штаммы *H. Pylori*.

Материалы и методы: Наночастицы золота синтезированы методом Туркевича (Turkevich). Затем их размер и дисперсию исследовали с помощью спектрофотометрии, DLS и TEM-микроскопии. Впоследствии комбинация метронидазола и наночастиц золота была достигнута методом смешивания, а затем антихеликобактерные эффекты этих двух препаратов были оценены в соответствии с требованиями Института клинических и лабораторных стандартов (CLSI).

Результаты: Самая высокая частота наночастиц составляла от 12 до 9 nm, а максимальное поглощение составляло 522 nm; однако в конъюгированном состоянии максимальное поглощение составляло 540 nm, что является индикатором накопления наночастиц, конъюгированных с лекарственным средством. Некоторые изменения свидетельствовали о связывании метронидазола с наночастицами золота. Антимикробное тестирование наночастиц золота и метронидазола не повлияло на *Helicobacter pylori*. Для этого комбинация наночастиц золота и метронидазола имела 17-миллиметровую зону задержки роста.

Заключение: Антихеликобактерные эффекты метронидазола были значительно усилены конъюгацией с наночастицами золота.

Ключевые слова

антимикробная активность, покрытый, *Helicobacter pylori*, наночастицы золота, метронидазол