ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2009, p. 1592–1597 Vol. 53, No. 4
0066-4804/09/$08.00+0 doi:10.1128/AAC.01242-08

Antimicrobial Activity of Curcumin against *Helicobacter pylori* Isolates from India and during Infections in Mice[▽]

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Received 18 September 2008/Returned for modification 29 October 2008/Accepted 28 January 2009

Treatment failure is a major cause of concern for the *Helicobacter pylori*-related gastroduodenal diseases like gastritis, peptic ulcer, and gastric cancer. Curcumin, diferuloylmethane from turmeric, has recently been shown to arrest *H. pylori* growth. The antibacterial activity of curcumin against 65 clinical isolates of *H. pylori* in vitro and during protection against *H. pylori* infection in vivo was examined. The MIC of curcumin ranges from 5 μg/ml to 50 μg/ml, showing its effectiveness in inhibiting *H. pylori* growth in vitro irrespective of the genetic makeup of the strains. The nucleotide sequences of the *aroE* genes, encoding shikimate dehydrogenase, against which curcumin seems to act as a noncompetitive inhibitor, from *H. pylori* strains presenting differential curcumin MICs showed that curcumin-mediated growth inhibition of Indian *H. pylori* strains may not be always dependent on the shikimate pathway. The antimicrobial effect of curcumin in *H. pylori*-infected C57BL/6 mice and its efficacy in reducing the gastric damage due to infection were examined histologically. Curcumin showed immense therapeutic potential against *H. pylori* infection as it was highly effective in eradication of *H. pylori* from infected mice as well as in restoration of *H. pylori*-induced gastric damage. This study provides novel insights into the therapeutic effect of curcumin against *H. pylori* infection, suggesting its potential as an alternative therapy, and opens the way for further studies on identification of novel antimicrobial targets of curcumin.

Helicobacter pylori is a microaerophilic bacterium with the extraordinary ability to establish infections in human stomachs that can last for years or decades. It is carried by more than one-half of all people worldwide, and its prevalence exceeds 90% in some developing countries like India. It has attracted great attention as a major cause of peptic ulcer disease. In fact, *H. pylori* is the first bacterium to be classified as a group I carcinogen by the International Agency for Research on Cancer (6, 31). Based on the genetic characteristics and disease outcome, there are significant geographic differences among *H. pylori* strains. Indian *H. pylori* strains are genetically distinct from those from east Asia and the West (4, 16). Several putative virulence-associated factors, including alleles in the *cag* pathogenicity island (PAI) of *H. pylori*, contribute to its pathogenesis and augment the risk for gastric adenocarcinoma (14). As virulence markers of *H. pylori* are not always associated with diseases, eradication of *H. pylori* from infected individuals remains the best choice for an effective treatment of *H. pylori*-associated diseases. Several combination therapies have been formulated to eradicate this pathogen and cure or prevent associated diseases. Triple therapy, consisting of the combined usage of two antibiotics and a proton pump inhibitor, gives a high eradication rate, producing a significant improvement in the status of the disease (30). However, eradication by the triple therapy is not always successful, and the acquisition by *H. pylori* of resistance to antibiotics, including metronidazole and clarithromycin, could represent a serious problem that may reduce treatment efficacy (10). Many studies have indicated that the prevalence of resistance varies geographically, ranging from 10 to 90% for metronidazole and from 0 to 15% for clarithromycin (7, 18, 30). In view of the incomplete cure achieved with conventional therapy because of increasingly resistant strains, undesirable side effects (17), noncompliance among the patients (3), the cost of the antibiotic regimens (32), and a few other factors contributing to ineffectiveness, there is an urgent need to develop new treatment strategies for *H. pylori* infection.

Previous studies have shown that the shikimate pathway is essential for the synthesis of important metabolites such as aromatic amino acids, folic acid, and ubiquinone (20). The enzymes involved in this pathway, including shikimate dehydrogenase (SDH), have recently gained great attention as novel drug targets for developing antimicrobial agents that are nontoxic (5). In support of this idea Han et al. (11) showed curcumin to be a noncompetitive inhibitor of SDH. This enzyme, encoded by the *aroE* gene of *H. pylori*, may provide useful information for treating *H. pylori*-associated infection.

In India since ancient times, spices and condiments have been considered indispensable in the culinary arts, as they are used to flavor foods. Also, these spices were recognized for their physiological and medicinal properties. Curcumin (diferuloylmethane), first chemically characterized in 1910, is generally regarded as the most active constituent of the perennial herb *Curcuma*

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In conclusion, our study highlights the potential antibacterial activity of curcumin against *H. pylori* in vitro, as curcumin was highly effective in inhibiting *H. pylori* growth irrespective of the genetic makeup of the strains, although its MIC is relatively high; this may be due to the poor bioavailability of curcumin (22). Our studies also showed that curcumin-mediated inhibition of *H. pylori* growth involved mechanisms that may not always be dependent on the shikimate pathway, which opens the way for further studies directed toward determination of novel antimicrobial targets of curcumin. Curcumin showed immense therapeutic potential against *H. pylori* infections and *H. pylori*-associated gastroduodenal diseases, as it was equally effective in eradicating *H. pylori* from infected mouse stomach. Moreover, the gastric damage induced by *H. pylori* infection was almost completely restored by curcumin, thus highlighting its potential as an alternative therapy against *H. pylori* infection. Overall, this study provides novel insights in the therapeutic potential of curcumin against *H. pylori* infections, although further studies are required to extrapolate its effect on humans.

فعالیت ضد میکروبی کورکومین بر علیه هلیکوباکتر پیلوری

هلیکوباکتر، یک باکتری با توانایی ایجاد عفونت در معده انسان می باشد و این بیماری می تواند سال ها گریبان گیر انسان باشد.

شکست در درمان هلیکوباکتر یکی از عوامل نگران کننده ایجاد بیماری هایی چون سرطان معده، گاستریت و زخم معده می باشد. در مطالعات توانایی آنتی باکتریال کورکومین نشان داده شده است. همچنین بیان می شود که کورکومین می تواند رشد هلیکوباکتر را متوقف کند. در مطالعات دیگر نیز توانایی کورکومین به منظور کاهش التهاب در معده در اثر ابتلا به هلیکوباکتر نشان داده شده است.

روش:

۶۵ گونه از هلیکوباکتر که از انسان نمونه گیری شده بودند در این مطالعه استفاده شدند.نمونه ها در BHI - آگار کشت داده شدند. تاثیر غلظت های mcg/ml ۵۰, ۴۰, ۳۰, ۲۵, ۲۰, ۱۵, ۱۰،۵ از کورکومین بررسی شد.

نتایج:

کورکومین هلیکوباکتر را در معده موش های مورد آزمایش از بین برد و نتایج هیستولوژی در تصویر زیر نشان داده شده است.

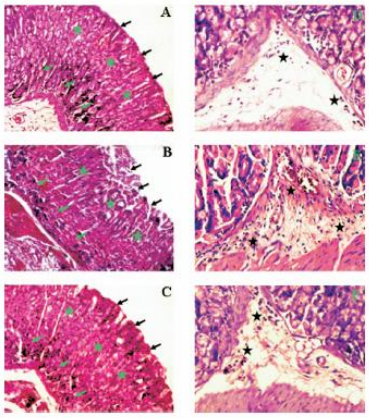

FIG. 5. Histology of control, *H. pylori*-infected, and curcumin-treated mouse gastric tissues. Histological sections were stained with hematoxylin and eosin, and photographs were taken at ×10 and ×20 magnifications, respectively. (A to C) Histological appearance of gastric tissues from (A) control mice, (B) mice infected with SS1 for 3 weeks, and (C) mice infected with SS1 and treated with curcumin at 10× magnification. (D to F) Higher-magnification (20×) views of (D) control, (E) SS1-infected, and (F) SS1-infected, curcumin-treated mouse gastric tissues. The gastric mucosal epithelium (black arrows), gastric pits (green stars), gastric glands (green arrows), and inflammatory cell infiltration (black stars) are shown.

نتیجه گیری:

در این مطالعه پتانسیل فعالیت ضد باکتریایی کورکومین در شرایط in-vivo نشان داده شد و با توجه به فراهمی زیستی پایین کورکومین MIC بسیار بالا بود. همچنین در این مطالعه، پس از مصرف کورکومین آسیب ایجاد شده در معده به طور کامل بهبود یافت.