Arch Pharm Res Vol 29, No 10, 879-883, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Trials of Novel ^{13}C-Urea-Containing Capsule for More Economic and Sensitive Diagnosis of *Helicobacter pylori* Infection in Human Subjects

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(Received July 7, 2006)

To develop a ^{13}C-urea-containing capsule for more economic and sensitive diagnosis of *Helicobacter pylori* infection, the ^{13}C-urea-containing capsules were prepared with various additives such as polyethylene glycol, microcrystalline cellulose, sodium lauryl sulfate and citric acid. Their dissolution test and ^{13}C-urea Breath Test in human volunteers were then performed. Polyethylene glycol increased the initial dissolution rates of urea and difference δ ^{13}C values from ^{13}C-urea, while microcrystalline cellulose and sodium lauryl sulfate decreased them. Irrespective of addition of citric acid, the compositions with polyethylene glycol showed higher values from ^{13}C-urea compared to a commercial 76 mg ^{13}C-urea-containing capsule due to higher initial dissolution rate. The capsules with 38 mg ^{13}C-urea and 1.9 mg polyethylene glycol, which showed higher *Helicobacter pylori*-positive value of about 8‰ at 10 min, improved the sensitivity of ^{13}C-urea in human volunteers. Thus, the ^{13}C-urea-containing capsule with polyethylene glycol would be a more economical and sensitive preparation for diagnosis of *Helicobacter pylori* infection.

Key words: *Helicobacter pylori*, Urea, Polyethylene glycol, Dissolution, ^{13}C-Urea breath test

INTRODUCTION

Helicobacter pylori, one of the commonest bacterial infection, may lead to gastritis, peptic ulceration, non-ulcer dyspepsia and possible gastric cancer (Faigel *et al.*, 1996; Grimley *et al.*, 1999). Eradication of *Helicobacter pylori* infection may lead to long-term remission of peptic ulceration and improvement of gastritis. Thus, it is obvious that the test detecting the bacterium is great clinical significance (Lerang *et al.*, 1998). The urea breath test with ^{13}C-urea offers a unique advantage as non-invasive, very sensitive, fast and highly specific method by comparison either laborious culture and histology. The urea breath test can detect *Helicobacter pylori* colonization within the stomach assessing the entire mucosa non-invasively avoiding the risk of sampling error or an observer bias. In contrast to serology, the urea breath test offers excellent diagnostic value as a post treatment control to confirm eradication of *Helicobacter pylori* (Bielanski and Konturek, 1996; Graham *et al.*, 2001; Parente and Bianchi, 2001; Prewett *et al.*, 1992; Savarino *et al.*, 1999). A commercial product, Helikit was formulated with only expensive 75 mg ^{13}C-urea administrated orally in a capsule form with or without previous test meal (Hamlet *et al.*, 1995; Hamlet *et al.*, 1999). The urea ingestion by this product may lead to false positive or negative results due to late diagnosis. Therefore, a new product for diagnosis with reduced test time and commercial cost of diagnosis must be needed (Graham *et al.*, 2001; Parente and Bianchi, 2001; Savarino *et al.*, 1999).

In this study, to develop a new ^{13}C-urea-containing capsule for more economic and sensitive diagnosis of *Helicobacter pylori* infection, the urea-containing capsules were prepared with various additives including dissolution-enhancing agent and test meal. Their dissolution test and ^{13}C-urea breath test in human volunteers were then performed. Throughout the experiments, the filling amount of ^{13}C-urea in the capsule was fixed as 38 mg, the half content of commercial product. Furthermore, polyethylene glycol (Rouchotas *et al.*, 2000; Stavchansky and Gowan,

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1984), microcrystalline cellulose (Yamamoto *et al.*, 1976) and sodium lauryl sulfate (Lee *et al.*, 2001) were used as dissolution-enhancing agents in the formulation of solid dosage forms such as tablet and capsule. Citric acid was used as a test meal in the diagnosis of *Helicobacter pylori* infection by ^{13}C-urea (Bielanski and Konturek, 1996; Hamlet *et al.*, 1995).

MATERIALS AND METHOD

Materials

Urea and ^{13}C-urea were purchased from MassTrace INC. (Mass., U.S.A.) and Sigma Chemical (St. Louis, MO, USA), respectively. Polyethylene glycol (PEG 4000), microcrystalline cellulose (Avicel PH 102), sodium lauryl sulfate and citric acid were of USP grade. All other chemicals were of reagent grade and used without further purification.

Preparation of urea-containing capsules

Urea, polyethylene glycol, microcrystalline cellulose, sodium lauryl sulfate and citric acid were passed through the sieve (106 μm), blended and filled in the capsules (size 0) equivalent to 380 or 760 mg urea per capsule. The detailed compositions of capsules are given in Table I.

Dissolution test

Various urea-containing capsules were placed in a dissolution tester (DST-600, Fine Chemical, Korea), respectively. Dissolution test was performed at 36.5°C using the paddle method at 50 rpm with 500 ml oL pH 1.2 (0.1 N HCl) as a dissolution medium. At predetermined interval, 1 ml of the medium was sampled and filtered. This resulting solution was directly injected (20 μL) onto an Inertsil ODS-3 C_{18} column (GL science, 0.5 mm, 15 cm × 0.46 cm i.d.). The chromatograph consisted of a high-performance chromatograph (Hitachi, Model L-7100) and a variable ultraviolet spectrophotometric detector (Model L-7450). The mobile phase consisted of acetonitrile and water (66:34, volume ratio). The eluent was monitored with UV/vis detector set at 280 nm with a flow rate of 1.0 mL/min (Choi *et al.*, 2000; Yamamoto *et al.*, 1976).

Table I. Compositions of ^{13}C-urea-containing capsules

Composition (mg/capsule)	A	B*	C	D	E	F	G
Urea	38	75	38	38	38	38	38
Microcrystalline cellulose	-	-	1.9	-	-	-	-
Sodium laurylsulfate	-	-	-	1.9	-	-	-
Polyethylene glycol	-	-	-	-	1.9	3.8	1.9
Citric acid	-	-	-	-	-	-	1.9
Total	38	75	39.9	39.9	39.9	41.8	41.8

*Composition B was a commercial product.

**Each amount of composite in all urea-containing capsules was 10-fold higher than that in ^{13}C-urea-containing ones, respectively.

^{13}C-urea breath test in human volunteers

Subject

Six patients, 25-33 years old, attending for routine upper gastrointestinal endoscopy entered the study, which was approved by the Yeungnam University Ethical Committee. All of them signed informed consent. The exclusion criteria for the study were; possible pregnancy, previous gastric surgery, recent use of medications such as bismuth or antibiotics (within 1 month) and/or sucralfate, proton pump inhibitors or histamine-receptor antagonists taken in the past 2 weeks. For each examination patients were asked to come after overnight fast.

Preparation of ^{13}C- urea-containing capsules

^{13}C-urea, polyethylene glycol, microcrystalline cellulose, sodium lauryl sulfate and citric acid were passed through the sieve, blended and filled in the capsules (size 2) equivalent to 38 or 76 mg ^{13}C-urea per capsule. The detailed compositions of capsules are given in Table I.

^{13}C-urea breath test

All consenting patients were tested at random order non-invasively for the presence of *Helicobacter pylori* false with the use of ^{13}C-urea. The fast breath sample was collected from each patient (prior to ^{13}C-urea administration) at baseline followed by ingestion of various capsules with 25 mL of water, respectively. After 3 min, additional 25 mL of water was drunk and breath samples were collected at the predetermined times. The breath samples collected into aluminized breath-bag (Teobag, 1.5 L, Tessaraux Container, Burstadt, Germany) were directly connected to isotope-selective non-dispersive infrared spectrometer (IRIS, Wagner Analysen Technik, Worpswede, Germany), which allowed continuous flow of at least 500 mL of air. The final results of $^{13}CO_2/^{12}CO_2$ ratios measured by the isotope-selective non-dispersive infrared-spectroscopy were expressed as δ $^{13}CO_2$ (per mil) values (Bielanski and Konturek, 1996; Hamlet *et al.*, 1995). These experiments were performed in six volunteers separated by a period of one week.

RESULTS AND DISCUSSION

To test whether various additives affect the dissolution rates of urea from the capsules, we performed the dissolution studies on the formulations composed of 38 or 76 mg urea, 1.9 mg of additives such as polyethylene glycol, microcrystalline cellulose and sodium lauryl sulfate

per capsule (Table I).

The dissolution of urea was affected by additives such as polyethylene glycol, microcrystalline cellulose, sodium lauryl sulfate (Fig. 1A-B). Polyethylene glycol (composition E) increased the initial dissolution rates of urea until 3 min, while microcrystalline cellulose (composition C) and sodium lauryl sulfate (composition D) delayed the initial dissolution rates of urea until 6 min followed by the similar dissolution rates to that from the capsule filled with urea alone (composition A). Their effects on the dissolution of urea from the capsules were possibly contributed by that alkalic urea molecules were absorbed not at polyethylene glycol (Stavchansky and Gowan, 1984; Rouchotas *et al.*, 2000) but at microcrystalline cellulose (Yamamoto *et al.*, 1976) and sodium lauryl sulfate (Lee *et al.*, 2001). The more amounts of urea, the less increased the dissolution rates of urea from the capsules (composition A vs. B).

To test whether citric acid, a test meal affects the dissolution rates of urea from the capsules, we performed the dissolution studies on the formulations composed of 380 mg urea, 19-38 mg polyethylene glycol, 19 mg citric acid per capsule (Table I). Fig. 1A indicated that, in the presence of polyethylene glycol, citric acid hardly affected the dissolution rates of urea (composition E vs. G). The increased amounts of polyethylene glycol, the less increased the dissolution rates of urea from the capsules (composition E vs. F). Furthermore, irrespective of addition of citric acid, the compositions with polyethylene glycol (composition E-G) showed higher initial dissolution rate of urea compared to a commercial urea-containing capsule (composition B).

The ^{13}C-urea breath test exploits the copious amounts of urease produced by *Helicobacter pylori*, which hydrolyses urea to form ammonia and soluble carbon dioxide expired in the exhaled breath. Labeling of urea with either isotope allows the $^{13}CO_2$ to be detected in the expired breath. ^{13}C is always measured as a ratio of ^{13}C to ^{12}C (δ $^{13}CO_2$ per mil), therefore the amount of excreted CO_2 does not need to be measured and an expired air suffices for analysis. When *Helicobacter pylori* are present, the relative amount of $^{13}CO_2$ increases considerably (Bielanski and Konturek, 1996; Graham *et al.*, 2001; Parente and Bianchi, 2001; Prewett *et al.*, 1992; Savarino *et al.*, 1999). A difference δ ^{13}C value over baseline for two time points after capsule ingestion, of more than 5‰ was considered as positive result (Eggers *et al.*, 1990; Logan, 1998; Peura *et al.*, 1996).

To test whether various additives affect the difference δ ^{13}C value from ^{13}C- urea-containing capsules, the ^{13}C-urea breath test on the formulations composed of 38 mg ^{13}C-urea, 1.9 mg of additives such as polyethylene glycol, microcrystalline cellulose and sodium lauryl sulfate per capsule was performed (Table I).

The difference δ ^{13}C value was affected by additives such as polyethylene glycol, microcrystalline cellulose, sodium lauryl sulfate (Fig. 2). Polyethylene glycol (composition E) increased the values, while microcrystalline cellulose (composition C) and sodium lauryl sulfate (composition D) decreased them. The reason for these effects of additives on the difference δ ^{13}C value was why polyethylene glycol increased the initial dissolution rates of urea, while microcrystalline cellulose and sodium lauryl sulfate delayed them. Furthermore, Composition A, C, D and E showed the *Helicobacter pylori*-positive value of 5‰ at 15, 20, 20 and 10 min, respectively, indicating that the capsule with polyethylene glycol (composition E) shortened greatly the duration time of diagnosis of *Helicobacter pylori* infection. Composition A, C, D and E

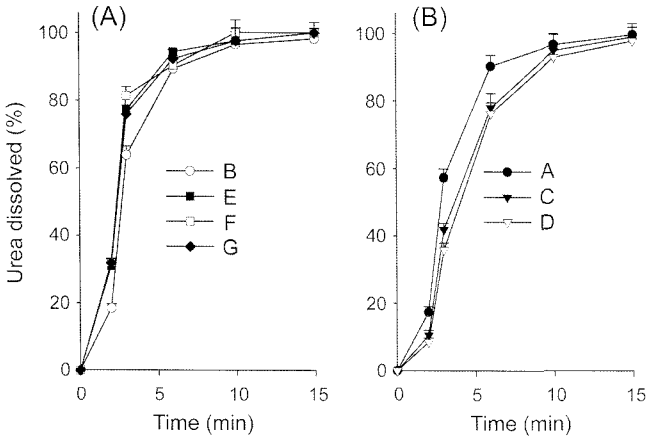

Fig. 1. Dissolution rates of urea from various capsules composed of urea, microcrystalline cellulose, sodium lauryl sulfate, polyethylene glycol and citric acid. Each value represents the mean±S.D. (n=6).

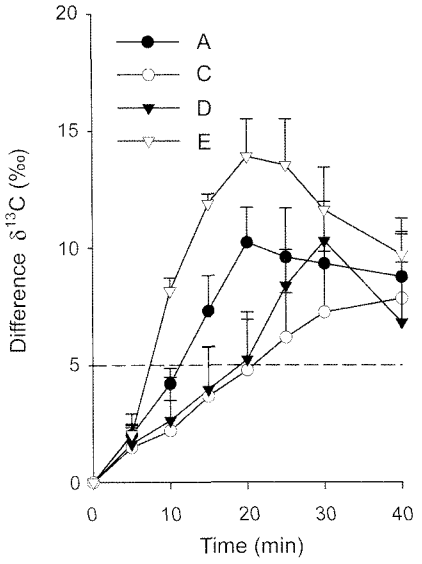

Fig. 2. Typical $^{13}CO_2$ exhalation curves in six human volunteers after oral administration of ^{13}C-urea-containing capsules composed of various additives. Each value represents the mean±S.D. (n=6).

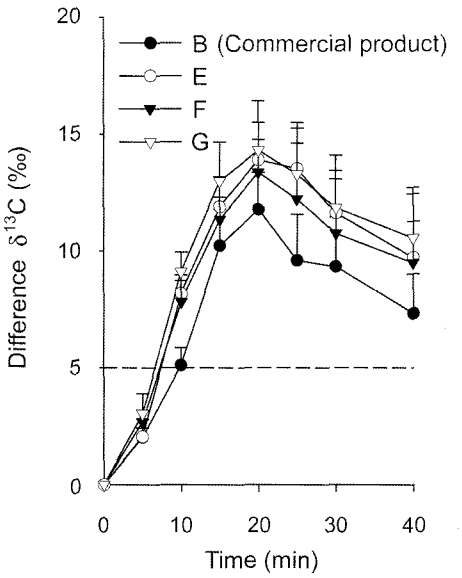

Fig. 3. Typical $^{13}CO_2$ exhalation curves in six human volunteers after oral administration of ^{13}C-urea-containing capsules composed of polyethylene glycol and citric acid. Each value represents the mean±S.D. (n=6).

showed the maximum value of about 10, 7, 10 and 14‰, respectively, indicating that only the capsule with polyethylene glycol (composition E) increased greatly the response of ^{13}C-urea against *Helicobacter pylori*. Therefore, polyethylene glycol was selected as a additive of ^{13}C-urea-containing capsule, since polyethylene glycol improved the sensitive diagnosis of *Helicobacter pylori* infection by ^{13}C-urea.

To test whether citric acid affects the difference δ ^{13}C value from ^{13}C- urea-containing capsules, the ^{13}C-urea breath test on the formulations composed of 38 mg ^{13}C-urea, 19-38 mg of polyethylene glycol, 19 mg citric acid per capsule was performed (Fig. 3). Citric acid as a test meal was reported to improve the sensitive diagnosis of *Helicobacter pylori* infection by ^{13}C-urea (Bielanski and Konturek, 1996; Hamlet *et al.*, 1995).

Citric acid increased slightly the difference δ ^{13}C values from the capsules (composition G vs. E in Fig. 1A), but there was no significant difference between them. The increased amount of polyethylene glycol, the less increased the difference δ ^{13}C values from the capsules (composition F vs. E). Compared to a commercial ^{13}C-urea-containing capsule (composition B), irrespective of citric acid, the compositions with polyethylene glycol (composition E-G) showed higher difference δ ^{13}C values from the capsules. Furthermore, all compositions showed the *Helicobacter pylori*-positive value of 5‰ at 10 min, indicating that, irrespective of citiric acid, the capsule with polyethylene glycol (composition E-G) had the same duration time of diagnosis to a commercial ^{13}C-urea-containing capsule (composition B). However, composition B, E, F and G showed the value of about 5, 8, 8 and 9‰ at 10 min, respectively, indicating that the capsule with polyethylene glycol (composition E-G) increased greatly the response of ^{13}C-urea against *Helicobacter pylori*. As a possible mechanism by which polyethylene glycol affected the diagnosis of *Helicobacter pylori* infection from ^{13}C-urea capsules, it is speculated that polyethylene glycol shortened the dissolution time of ^{13}C-urea-containing capsule in the gastric tract, leading to increasing difference δ ^{13}C value (Rouchotas *et al.*, 2000; Stavchansky and Gowan 1984).

CONCLUSION

It is concluded that the capsules with 38 mg ^{13}C-urea and 1.9 mg polyethylene glycol, which showed higher *Helicobacter pylori*-positive value of 8‰ at 10 min, improved the sensitivity of ^{13}C-urea in the human volunteers. Thus, the ^{13}C-urea-containing capsule with polyethylene glycol would be a more economical and sensitive preparation for diagnosis of *Helicobacter pylori* infection.

ACKNOWLEDGEMENTS

This research was supported by the Yeungnam University research grants in 2006.

REFERENCES

Bielanski, W. and Konturek, S. J., New approach to ^{13}C-urea breath test: capsule-based modification with low-dose of 13C-urea in the diagnosis of Helicobacter pylori infection. *J. Physiol. Pharmacol.*, 47, 545-553 (1996).

Choi, H. G., Yong, C. S., and Kim, C. K., Development of terfenadine-pseudoephedrine double layered tablet dissolution-equivalent to core tablet. *Drug Dev. Ind. Pharm.*, 26, 605-611 (2000).

Eggers, R. H., Kulp, A., Tegeler, R., Ludke, F. E., Lepsien, G., Meyer, B., and Bauer, F. E., A methodological analysis of the (^{13})C-urea breath test for detection of Helicobacter pylori infections : high sensitivity and specificity within 30 min using 75 mg of (^{13})C-urea. *Eur. J. Gastroen. Hepat.*, 2, 437-444 (1990).

Faigel, D. O., Childs, M., Furth, E. E., Alavi, A. and Metz, D. C., New noninvasive tests for Helicobacter pylori gastritis. Comparison with tissue-based gold standard. *Digest. Dis. Sci.*, 41, 740-748 (1996).

Graham, D. Y., Malaty, H. M., Cole, R. A., Martin, R. F., and Klein, P. D., Simplified ^{13}C-urea breath test for detection of Helicobacter pylori infection. *Am. J. Gastroentero.*, 96, 1741-1745 (2001).

Grimley, C. E., Penny, A., O'sullivan, M., Shebani, M., Lismore, J. R., Cross, R., Illing, R. C., Loft, D. E., and Nwokolo, C. U.,

Comparison of two 3-day Helicobacter pylori eradication regimens with a standard 1-week regimen. *Aliment Pharm. Therap.,* 13, 869-873 (1999).

Hamlet, A. K., Erlandsson, K. I., Olbe, L., Svennerholm, A. M., Backman,V. E., and Pettersson, A. B., A simple, rapid, and highly reliable capsule-based ^{14}C urea breath test for diagnosis of Helicobacter pylori infection. *Scand. J. Gastroentero.,* 30, 1058-1063 (1995).

Hamlet, L., Stage, H., Lonroth, C., Cahlin, Nystrom, C., and Pettersson, A., A novel tablet-based ^{13}C urea breath test for Helicobacter pylori with enhanced performance during acid suppression therapy. *Scand. J. Gastroentero.,* 34, 367-374 (1999).

Lee, E. J., Lee, S. W., Choi, H. G., and Kim, C. K., Bioavailability of cyclosporin A dispersed in sodium lauryl sulfate-dextrin based solid microspheres. *Int. J. Pharm.,* 218, 125-131 (2001).

Lerang, F., Haug, J. B., Moum, B., Mowinckel, P., Berge, T., Ragnhildstveit, E., and Bjorneklett, A., Accuracy of IgG serology and other tests in confirming Helicobacter pylori eradication. *Scand. J. Gastroentero.,* 33, 710-715 (1998).

Logan, R. P. H., Urea breath tests in the management of Helicobacter pylori infection, *Gut,* 43, S47-S50 (1998).

Parente, F. and Bianchi, P. G., The (^{13})C-urea breath test for non-invasive diagnosis of Helicobacter pylori infection: which procedure and which measuring equipment? *Eur. J. Gastroen. Hepat.,* 13, 803-806 (2001).

Peura, D. A., Pambianco, D. J., Dye, K. R., Lind, C., Frierson, H. F., Hoffman, S. R., Combs, M. J., Guilfoyle, E., Marshall and B. J., Microdose ^{13}C-urea breath test offers diagnosis of Helicobacter pylori in 10 minutes. *Am. J. Gastroenterol.,* 91, 233-238 (1996).

Prewett, E. J., Luk, Y. W., Fraser, A. G., Lam, W. M. and Pounder, R. E., Comparison of one-day oral dosing with three bismuth compounds for the suppression of Helicobacter pylori assessed by the ^{13}C-urea breath test. *Aliment Pharm. Therap.,* 6, 97-102 (1992).

Rouchotas, C., Cassidy, O. E., and Rowley, G., Comparison of surface modification and solid dispersion techniques for drug dissolution. *Int. J. Pharm.,* 195, 1-6 (2000).

Savarino,V., Vigneri, S., and Celle, G., The ^{13}C urea breath test in the diagnosis of Helicobacter pylori infection. *Gut,* 45, S118-S122 (1999).

Stavchansky, S. and Gowan, W. G., Evaluation of the bio-availability of a solid dispersion of phenytoin in polyethylene glycol 6000 and a commercial phenytoin sodium capsule in the dog. *J. Pharm. Sci.,* 73, 733-736 (1984).

Yamamoto, K., Nakano, M., Arita, T., Takayama, Y., and Nakai, Y., Dissolution behavior and bioavailability of phenytoin from a ground mixture with microcrystalline cellulose. *J. Pharm. Sci.,* 65, 1484-1488 (1976).