Antimicrobial susceptibility testing method using surface plasmon resonance
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Surface plasmons was first predicted by Ritchie in 1957 and demonstrated by Otto using optical excitation in 1968. For the past two decades, SPs excited by light has been widely applied to the study of biomaterial processes, including biosensors, immunodiagnostics, and kinetic analysis of antibody–antigen interaction. The main application of surface plasmon resonance, SPR, on the biomedical science is to analyze the binding dynamics between antibody and antigen and to sense specific antigen through the chemical binding of corresponding antibody. Although dynamic analysis is required for measuring affinity of cognate molecules, most of the applications of SPR so far can be considered as stationary measurement. It is only used on detection or recognition of cognate targets or development of antibodies. Until recently, using SPR biosensor on detecting whole cells was explored. These efforts include an observation of cell/substrate contacts, a reaction of cells to toxicity, and detection of cholesterol concentration in cell membranes.

Infectious diseases are a leading cause of morbidity and mortality in hospitalized patients. This fact places a tremendous burden on the clinical microbiology laboratory to rapidly diagnose the agent responsible for patient’s infection and to effectively provide therapeutic guidance for eradication of the organism. In agriculture, rapid diagnosis of drug resistance of bacteria also has enormous impact on agriculture economy. Besides, laboratories are asked not only to perform these tasks with efficiency, but also in a cost effective manner in an era of increasing emphasis on reduction of laboratory expenses. Two common methodologies for antimicrobial susceptibility testing in a clinical laboratory are Kirby-Bauer disk diffusion and variations of broth microdilution. These methods usually take from 1 day to weeks to complete the experiments and issue the reports. Such a waiting period is not short for patients and clinical doctors who urgently need the information to adjust the therapeutic strategy.

To our best knowledge, this paper is the first effort to detect the dynamical change of bacteria to antibiotics by SPR biosensor. While current antimicrobial susceptible testing methods execute antimicrobial test based on the detection of cell duplication ability under the influence of antibiotics, the SPR biosensing method directly detect chemical or physical change of bacteria subject to antibiotics. This research then focused on exploring new application of SPR biosensing technique on antimicrobial susceptibility test. It is expected to take much shorter time than the conventional methods do. Since SPR is highly sensitive to the change of the refractive index of cells near the cell–metal interface, ampicillin as the antibiotic inhibiting the synthesis of cell walls was used for the examination of E. coli JM109.
Since SPs penetrate into cells approximately in the order of 100 nm, the biosensor is very sensitive to the change of the refractive index of cell walls and membranes attached on the Au thin film within that range – no matter what causes the change. The mechanism of ampicillin is to interrupt the activity of transpeptidase, which assists the formation of peptide bond combined with peptidoglycan to constitute cell walls, and then to interfere cell growth and proliferation. Therefore, for the measurement of direct influence of antibiotics, ampicillin was used as the antibiotic for the antimicrobial susceptibility test. *E. coli* JM109 strains were used as the tested objects.

To test the drug resistance of *E. coli* JM109 using the SPR system, sterilized DI water was injected into the cell chamber for 30 min to stabilize the system after the biochip coated with Poly-l-lysine was assembled. Following the stabilization procedure, the incubated LA broth was injected into the fluidic cell chamber for about 60 min to cover the Au thin layer. Another wash procedure was followed to remove the bacteria that were not bound to the Poly-lysine layer. When the bacteria binding reached a steady state, the ampicillin solution with concentration 3 μg/ml was injected. The resonance angle of surface plasmon through the entire procedure was recorded as a function of time.

![Fig. 1 Kinetic plot of SPR angle shift for the antimicrobial susceptibility test of *E. coli* to ampicillin for 30 min: (a) angle shift of the ampicillin-resistant strain is around −0.00154° after the treatment for 30min; (b) angle shift of the ampicillin-susceptible strain is around −0.01608° after the treatment for 30min.](image)

The SPR angle of antibiotic-resistant strain of *E. coli* JM109 over the operation procedure described above is shown in Fig. 1(a) and that of antibiotic-susceptible strain is shown in Fig. 1(b). The shift of the SPR angle was referred to the value of the SPR angle before the *E. coli* strain was injected into the cell chamber. As is shown in Fig. 1(a), the SPR angle increased when the bacteria were injected into the cell chamber. After the amount of the bacteria attached to the Au thin film coating was saturated, DI water was injected to remove the unbound bacteria. The SPR angle dropped dramatically during this procedure. After bacteria binding reached steady state, the 3 μg/ml ampicillin was injected into the cell chamber. The value of SPR angle, changed by the refractive index of the bacterium surface, was recorded over time. The same procedure was applied on the susceptible strain and the result is shown in Fig. 1(b). The result shows that, after 30min treatment of ampicillin, the decrease of the SPR angle for the resistant and the susceptible strains were −0.00154° and −0.01608°, respectively. The angle shift is about 10 times difference between the resistant strain and the susceptible strain. The decrease of the refractive index, which is depicted by the negative angle shift, is possible from the structure loose of bacteria cell walls or even breakdown due to the effect of ampicillin. Since the antibiotic-resistant strain is more resistant to ampicillin, the refractive index of its cell walls did not decrease as much as that of the susceptible strain.
Fig. 2. SEM scanning pictures: (a) *E. coli* without the treatment of ampicillin; (b) ampicillin-resistant strains after 30-min treatment of ampicillin; (c) ampicillin-susceptible strains after 30-min treatment of ampicillin.

The damage degree of the ampicillin on the cell walls of the strain susceptible to ampicillin was examined by scanning electron microscope (SEM). The *E. coli* before the treatment of the ampicillin is shown in Fig. 2(a). The antibiotic resistant and susceptible *E. coli* strains after the antibiotic treatment for 30min are shown in Fig. 2(b) and (c), respectively. A comparison between the SEM pictures revealed no significant change in the appearance of the resistant strain and that of the susceptible strain subject to the treatment of ampicillin for 30 min. However, after 5 h of treatment of ampicillin, the susceptible strain shrank, which was verified by SEM.

We have reported an innovative antimicrobial susceptible testing method utilizing SPR. Susceptible and resistant strains of *E. coli* (Gram-negative) JM109 to ampicillin were examined. Since ampicillin directly acts on the formation of cell walls, the susceptible strain of *E. coli* revealed monotonic decrement over time on the SPR angle. The experimental result has shown the feasibility of utilizing SPR biosensors for fast antimicrobial susceptibility test. Quantitative analysis of drug resistance has to be studied and established.
Effects of inorganic nutrient levels on the biodegradation of benzene, toluene, and xylene (BTX) by \textit{Pseudomonas spp.} in a laboratory porous media sand aquifer model

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Introduction

The appearance of BTX compounds in natural environments is usually associated with the spill or discharge of petroleum products and synthetic chemicals in the form of herbicides, pesticides, and industrial effluents\textsuperscript{[1-2]}. There is considerable interest on adverse health effects and cancer-risk levels for exposure to various BTX compounds. Mehlman\textsuperscript{[3]} reported significant increase in tumors of kidney, liver and other tissues and organs following exposure to gasoline. Bioremediation of BTX-contaminated soil and groundwater by indigenous bacteria has received great attention in recent year\textsuperscript{[4]}. The biodegradation kinetics depends upon the addition of substrates, which can enhance the growth of BTX degraders. The use of substrates to enhance BTX degradation by indigenous bacteria has proved to be a promising strategy to remediate polluted aquifers. The main objectives of this study are to understand (1) the biodegradability of the benzene, toluene, and xylene (BTX) by \textit{Pseudomonas spp.} with amendments of varying amounts of nutrients including sulfate (MgSO\textsubscript{4}7H\textsubscript{2}O), phosphate (KH\textsubscript{2}PO\textsubscript{4}), and ammonium chloride (NH\textsubscript{4}Cl), and (2) bacterial morphological changes due to the fluctuation in nutrient levels and biomass. The laboratory biodegradation experiments were conducted in a sand tank microcosm simulating BTX-contaminated aquifer conditions.

Material and methods

In this study, a glass tank of size 110 cm\texttimes26 cm\texttimes27 cm was constructed to simulate a semi-confined aquifer in which BTX degradation could be measured. To minimize volatilization of BTX, the top of the silt was sealed with a glass cover. The volatilization and re-adsorption of BTX in the aquifer layer was measured in the blank test using a cylinder. To quantify the effects of change in nutrient levels on BTX degradation, we performed different sets of experiments by varying concentrations on one of three inorganic nutrients (MgSO\textsubscript{4}7H\textsubscript{2}O, KH\textsubscript{2}PO\textsubscript{4} and NH\textsubscript{4}Cl). For instance, while MgSO\textsubscript{4}7H\textsubscript{2}O was added in different concentrations (2.5, 7.5, 10 and 20 mg/l) to test the BTX degradation efficiency, the concentrations of KH\textsubscript{2}PO\textsubscript{4} (650 mg/l) and NH\textsubscript{4}Cl (25 mg/l) remained unchanged. Benzene, toluene, and xylene were obtained saturated in the de-ionized water and considered as the sole carbon source. BTX concentrations were analyzed using gas chromatograph (initial concentration of BTX 15 mg/l). Sulfate, phosphate, and chloride ions were analyzed by ion chromatography. The bacteria used in this study were enriched from activated sludge obtained from the sewage treatment plant of the China Petroleum Company Refinery in Kaohsiung, Taiwan. The morphology of the bacteria was examined using a high-resolution scanning electron microscope (SEM) immediately after sampling from the sandy medium. The 16S rRNA gene sequences of the two bacterial isolates were aligned with those of the reference strains with more than the 98\% nucleotide similarity,
revealing that the isolates are *Pseudomonas spp.* including *Pseudomonas acephalitica* (AM407893), *Pseudomonas nitroreducens* (EF107515), *Pseudomonas multiresinivorans* (X96487), and *Pseudomonas azelaica* (AM088475).

**Results and discussion**

**Biodegradation experiments**

The experiments on BTX degradation by *Pseudomonas spp.* were performed at different nutrient levels. The results indicate that an increase in the concentration of nutrients would promote BTX degradation. When the nutrient concentrations were in the lower range (i.e., 2.5 mg/l of sulfate, 250 mg/l of phosphate, and 10 mg/l of ammonium chloride), the bacterial growth and BTX degradation were limited. The effect of ammonium chloride level on the biodegradation rates of BTX was less pronounced than those of sulfate and phosphate. Significant biomass increases were observed with an increase in the concentrations of phosphate or sulfate, whereas there was little effect on bacterial growth with the amendment of ammonia chloride. The system experienced the largest bacterial population growth at the highest phosphate concentration of 1250 mg/l and the least bacterial growth at a low phosphate concentration of 250 mg/l or a low sulfate concentration of 2.5 mg/l. Comparison of experimental results show that toluene appears to be the most biodegradable, followed by benzene and xylene; this result is also consistent with earlier reports. Sulfate and phosphate not only represent key nutrients for the growth of *Pseudomonas spp.*, they also serve as electron acceptors in the microbiological processes degrading BTX.

**Change in bacteria morphology during biodegradation**

Changes in temperature in the simulated semi-confined aquifer might affect the bacterial morphology during BTX biodegradation. Filamentous bacteria were the predominant population after 35 h of biodegradation at 20–25°C (Fig. 1) when the temperature reached 25-28°C, the filamentous bacterial cells were replaced by the rod-shaped and spherical cells. When the sulfate concentration decreased to 2.5 mg/l, the rod-shaped cells were not clustered, yielding lower biodegradation efficiency and microbial biomass (Fig. 2A). However, when the sulfate concentration increased to 20 mg/l, the small rod-shaped cells were clustered, resulting in higher biodegradation activity (Fig. 2B). If the sulfate concentration was in the range of 7.5 and 10 mg/l, the long and small rod-shaped cells became dominated, respectively (Figs. 2C-D). When the BTX mixture was allowed to be biodegraded at phosphate concentration of 650 mg/l for 21 h, high biomass of rod-shaped cells with lesser amounts of fine filamentous cells were present (Fig. 3A). When the phosphate concentration increased to 900 mg/l, many large filamentous cells and small amounts of fine filamentous cells were formed (Fig. 3B). Moreover, when the incubation time increased from 21 h to 42 h (at phosphate concentration 650 mg/l and 900 mg/l), the small rod-shaped cells were more clustered and became the dominant population. These results are consistent with the observations by Sinha and Annachhatre. Furthermore, the shape of the bacteria became much longer at higher concentration of 900 mg/l (Figs. 2C-D). The biodegradation efficiency also increased with increasing phosphate concentrations. In contrast, a change in ammonia chloride concentration did not significantly affect bacterial biomass or morphology during biodegradation.
The changes of bacterial morphology during the BTX biodegradation reveal that the filamentous cells were predominant at relatively low temperatures about 20°C during the early period, whereas the rod-shaped and spherical cells became dominant at higher temperatures ranging from 25-28°C during the late stage. The type and quantity of electron acceptors appear to control the morphology of *Pseudomonas spp.* biomass (Figs. 2 and 3), which in turn affects the biodegradation rates of BTX. At a high concentration of sulfate (20 mg/l), the smaller rod-shaped cells were clustered to degrade more BTX (Fig. 2B). At a high concentration of phosphate (900 mg/l), it was observed that filamentous cells (many large and a few small) were dominant (Fig. 3B).

It appears that an increase in sulfate concentration has the most pronounced effects on benzene degradation, whereas an increase in phosphate concentration tends to favor the biodegradation of benzene and xylene. Although the relationship among cellular forms, biological functions, and nutrients uptake remains poorly understood, it is likely that the morphological adaption is partly controlled by the nutrient levels in an environment where *Pseudomonas spp.* thrives. The long and thin cells have a favorable shape for maximizing contact with diffusing nutrients while minimizing increases in both surface area and cell volume. Many bacteria can accelerate or delay cell division when the nutrient levels in the environment fluctuate, thereby creating shorter or longer cells, respectively.[7] The microbial biomass also adjusted with time as nutrient levels change.

**Conclusions**

Biodegradation of BTX by *Pseudomonas spp.* with amendments of inorganic nutrients was studied at temperatures of 20-28°C and pH of 7-7.5 in a sandy medium. The experimental results indicate that BTX compounds could be effectively removed when the optimal concentrations of phosphates (650-1250 mg/l), ammonia chloride (10-50 mg/l), and sulfates (10-20 mg/l) were amended into the simulated aquifer. However, when the added concentrations were less than 250 mg/l, 10 mg/l, and 2.5 mg/l, respectively, the bacterial growth and BTX degradation became limited. An increase in ammonium concentration did not significantly enhance BTX degradation.

SEM studies were conducted to assess the influence of the different environmental parameters (temperature, nutrient levels, and incubation time) on microbial morphology. The bacteria were not clustered when the temperatures were low. The filamentous cells were dominant at 20°C after 35h, whereas they were replaced by the small rod-shaped and spherical clusters as the temperature increased from 25°C to 28°C. As the sulfate concentration was reduced to 2.5 mg/l, the rod-shaped cells were not clustered, yielding lower biodegradation efficiency and microbial biomass.
However, when the sulfate concentrations increased to 20 mg/l, the small rod-shaped cells became clustered, resulting in higher biodegradation efficiency of BTX. As the phosphate concentrations increased from 250 to 1250 mg/l, the biodegradation efficiency of BTX mixture also increased. This study demonstrates that the morphological adaptation of *Pseudomonas spp.* is strongly influenced by temperature and nutrient levels.

**References**

The impact of customer interface quality, satisfaction and switching cost on e-loyalty: Internet experience as a moderator

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ABSTRACT

The proliferation of the Internet has allowed businesses to offer their products and services through web-based applications, and it has undermined their ability to retain their customer. There is little doubt that the Internet will become an important channel for retailing. However, in e-commerce, it is difficult to build customer loyalty because of the low switching costs. Accordingly, both marketing academics and professionals have attempted to uncover the most critical factors leading to customer loyalty. In the context of e-commerce, customer interface functions as a salesperson that provides information to customers. Hence, researchers indicate that an appropriate customer interface is one of the critical factors for success in e-commerce. However, despite the recognition that the quality of the customer interface can influence consumers' purchase intention, the impact of customer interface quality on customer loyalty has essentially been ignored. Furthermore, although switching costs and customer satisfaction have been identified as major factors affecting customer loyalty in e-commerce businesses, few studies have investigated whether customer satisfaction and switching costs are related to customer interface quality. Specifically, the impacts of customer interface quality on satisfaction, switching costs and e-loyalty have not been examined for customers with different levels of Internet experience.

To fill these research gaps, the purpose of this study is to examine whether perceptions of customer interface quality affect customer loyalty in e-commerce and whether this effect is mediated by customer satisfaction and switching costs. This study also intends to investigate whether consumers' Internet experience moderates the relationships among customer interface quality, customer satisfaction, switching costs and e-loyalty.

This study proposes an integrating conceptual framework (see Figure 1), which adopted the cognitive-to-action loyalty phases of Oliver (1997; 1999) as a reference model, with the aim of focusing and elaborating on the relationships among customer interface quality, satisfaction, switching costs and e-loyalty. Notably, based on literature review and related studies, this study argues that the relationships among these constructs are moderated by Internet experience. Furthermore, based on a literature review, six hypotheses associated with the model have been generated, as follows:
Customer interface quality is a multi-faceted concept with several components. This study focuses on articulating the most prominent set of customer interface design features derived from numerous previous studies. Hence, this study adopts four dimensions to model customer interface quality, namely customization, interactivity, convenience and character. Customization is defined as the ability of a website to enable customers to access services tailored to their individual needs. Interactivity refers to the degree of interaction or dialogue between the website and the customer. Convenience is defined here as the ease of navigation and user-friendliness associated with a website. Character is defined as the aesthetic and functional look-and-feel of a website, achieved through the use of inputs such as text, style, graphics, colors, logos, and multimedia. Furthermore, the concept of e-loyalty extends the traditional concept of loyalty to online consumer behavior. Based on the study of Anderson and Srinivasan (2003) in the context of e-commerce, this study defines e-loyalty as a favorable customer attitude toward the e-store that predisposes the customer to repeat buying behavior. Based on the studies, this study suggests that perceived customer interface quality is a cognitive construct and assumes that customer interface quality directly affects e-loyalty. Thus, this study hypothesizes that:

**H1. Websites exhibiting a high degree of (a) customization, (b) interactivity, (c) convenience, and (d) character are associated with higher e-loyalty.**

Satisfaction is an affective response to purchase situations (Babin & Griffin, 1998; Bagozzi, Gopinath, & Nyer, 1999). This study treats customer satisfaction as cumulative and follows Anderson and Srinivasan (2003) in defining customer satisfaction as the level of customer contentment regarding prior purchasing experience with a specific website. Satisfaction with e-stores, like satisfaction with traditional stores, is not derived solely from customer satisfaction with the product purchased (Balabanis et al., 2006). Szymanski and Hise (2000) identify
convenience and site design as the main determinant of e-store satisfaction, which in turn influences the decision to repatronize a website. Moreover, loyal customers are not necessarily satisfied customers, but satisfied customers tend to be loyal customers (Fornell, 1992). Satisfying customers should thus be of extreme importance to e-stores in their efforts to keep customers loyal. Customers who are satisfied with the e-stores they currently use develop a positive attitude towards those e-stores. Thus, this study hypothesizes that:

H2. Websites exhibiting a high degree of (a) customization, (b) interactivity, (c) convenience, and (d) character are associated with higher customer satisfaction.

H3. Higher customer satisfaction is associated with higher e-loyalty.

Switching costs is defined here as consumer perceptions of the time, money, and effort associated with changing from one website to another. Switching costs can increase consumer dependence on an existing provider (Morgan & Hunt, 1994). In e-commerce, high switching costs are assumed to make it costly for customers to take their business to other websites with better offers. In a study of online search behavior, Adamic and Huberman (2001) find that the top 1% of websites capture 50% of all web traffic, indicating that shoppers limit their search to just a few popular sites. The most reasonable explanation for this phenomenon is that consumer search costs associated with shopping across e-stores increase with the number of competing alternatives (Bergen, Dutta, & Shugan, 1996), and an increase in the number of available alternatives at a single e-store can markedly reduce the opportunity costs of time, inconvenience and search expended in e-store hopping (Srinivasan et al., 2002). Hence, this study asserts that online customers tend to keep on using whatever websites are perceived as having high quality customer interfaces. These positive perceptions, in turn, are related to high switching costs. Moreover, Switching costs are important moderators of the relationship between customer satisfaction and customer loyalty (Lee et al., 2001; Wangenheim, 2003). Jones et al. (2000) and Caruana (2004) both find evidence of an interaction between switching costs and customer satisfaction that influences customer loyalty. Thus, the following hypothesis is derived:

H4. Websites exhibiting a high degree of (a) customization, (b) interactivity, (c) convenience, and (d) character are associated with higher switching costs.

H5. Switching costs moderates the relationship between customer satisfaction and e-loyalty.

Internet experience is defined here as having such a general experience by using a browser, such as Netscape or Internet Explorer, to access World Wide Web (WWW) pages. Researchers have observed that those who shop online tend to be those who have experience on the Internet (Blake & Neuendorf, 2003; Citrin et al., 2000). Since users with more Internet experience have more experience with the interfaces provided by interactive applications, they should be better able to exploit website offerings than inexperienced users. Consequently, this study argues that Internet experience moderates the relationships between customer interface quality and customer satisfaction, switching costs and e-loyalty, respectively. Thus, this study hypothesizes that:

H6. Internet experience moderates the influence of (a) customer interface quality on customer satisfaction; (b) customer interface quality on e-loyalty; and (c) customer interface quality on switching costs.

This study conducted a Web-based survey. Our research subjects were people who had been doing online shopping for a year or longer. The main survey had 334 questionnaires for analysis. Approximately, 51% of the respondents were male. 88% were aged between 20 and 40; 90% were educated to institute/college level and above; 45% were students and 53% were workers; 50% use the Internet more than 19 hours each week. The top four online shopping
websites were YAHOO/Kimo.com (110), YAHOO auction (88), PChome.com (40), Books.com (35). Empirical analyses are performed using structural equations modeling analysis.

The results of this study support the view that customer interface quality is an important antecedent of customer satisfaction and e-loyalty (H1, H2). Although no significant relationship was found between customer interface quality and switching costs, this study confirms that customer interface quality appears to positively influence switching costs when customers have high Internet experience (H4). This result also confirms the moderating role of Internet experience among the relationships of customer interface quality, customer satisfaction and switching costs (H6). Additionally, our findings show that satisfaction can play a crucial intervening role in the relationship between customer interface qualities and e-loyalty (H3). Consistent with previous studies, this study demonstrated that switching costs moderate the relationship between customer satisfaction and e-loyalty (H5).

CONTRIBUTION

This study contributes to the understanding of re-patronage behavior in online shopping by shedding light on some important issues related to the creation of customer loyalty that have not been addressed by previous studies. In conclusion, this study contributes to practice and research in multiple ways. First, a contribution of this exploratory study is the proposal of a conceptual model as a framework to guide the investigation of Internet patronage behavior. This study demonstrates that customer interface quality is a critical influence on the establishment of e-loyalty. Notably, convenience directly contributes to the generation of e-loyalty. It implies that if a website that is logical and convenient to use facilitates fast completion of a transaction, it is likely that customers will come back, particularly for those goal-oriented shoppers who are busy and more interested in ease of access and information about products and customer service. Hence, this study suggests that online firms should consider focusing more on customer interface design in their marketing strategies, particularly with regards to the ease of navigation and user-friendliness features.

Second, the results of this study show that customization, interactivity and character indirectly affect e-loyalty through customer satisfaction. Researchers indicate that website interface provides cues in the context of online environment and triggers customers' emotional responses, especially for experiential shoppers who are motivated by recreation. Specifically, this study shows that character (e.g. websites' text, style, graphics, colors, logos, and multimedia) exerts the strongest direct effect on the customers' level of satisfaction. This finding is particularly important for websites' managers when they decide how to allocate resources in designing their website interface. Online firms could invest substantially in digital imaging and multimedia technology to ensure that all images of products on their website are presented using high quality graphics and multimedia, which will arouse customers' positive emotions such as enjoyment and satisfaction, and in turn, enhance e-loyalty.

Furthermore, this study finds that customer interface quality positively affects switching costs only when customers have high Internet experience. Accordingly, this study suggests that marketing strategies for retaining high Internet experience customers should be based on attempts to increase perceptions of switching costs by designing convenience, character, interactivity, and customization into their website interface in order for customers to perceive these benefits.

Finally, the findings also confirms the research of Nysveen and Pedersen (2004), which posits that Internet experience is an important moderating variable in understanding customers' behavior in e-commerce. Hence, the results of this study provide encouraging empirical support for the hypothesized moderating effect of Internet experience on the relationships among customer interface quality, satisfaction and switching costs.
Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B

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An estimated 400 million people worldwide are chronically infected with hepatitis B virus (HBV). More than one-half of reported cases with hepatocellular carcinoma (HCC) and approximately one-third of reported cases of cirrhosis are caused by chronic hepatitis B. HCC and cirrhosis, in turn, result in approximately one million deaths each year. Chronic hepatitis B (CHB) patients with an elevated viral load have the highest risk of progressing to these life-threatening complications. To avoid or minimize liver disease progression, current CHB treatment recommendations now stress the importance of long-term maintenance of HBV DNA suppression. Long-term safety and efficacy data for antiviral medications are indispensable to guide the risk-benefit assessment for a patient.

Approved agents available for the treatment of CHB can be divided into two groups: interferons and nucleoside/nucleotide analogues (NAs). Treatment with standard or pegylated interferon has been shown to result in durable serologic responses (HBe seroconversion) in HBeAg-positive patients, but these therapies are limited by response (seroconversion) rates of between 20 and 40%, injectable administration, and a high incidence of adverse events.

NAs include lamivudine, adefovir dipivoxil (ADV), entecavir, telbivudine, and tenofovir. Lamivudine and telbivudine have demonstrated efficacy and long-term safety, but the benefits of treatment diminish as resistance after long term therapy. The most recently published CHB treatment recommendations now classify lamivudine and telbivudine as not preferred due to its high rate of resistance. Published data on the long-term efficacy and safety of adefovir and entecavir in HBeAg-positive patients are limited.

The current report presents efficacy results through week 240 for all patients who received ADV 10 mg during year 1 of the study, and safety results for the subset of patients who were enrolled in the long-term safety and efficacy study (LTSES) and received up to 5 years of ADV. However, during year 2, study medication had been misallocated during the second year of the study due to an error in the interactive voice response system used to assign blinded treatment. Sixty-five patients given ADV in year 1 elected to continue in a long-term safety and efficacy study (LTSES). At enrollment, the 65 LTSES patients were a median 34 years old, 83% male, 74% Asian, 23% Caucasian, median baseline serum hepatitis B virus (HBV) DNA 8.45 log_{10} copies/mL, and median baseline alanine aminotransferase (ALT) 2.0 x upper limit of normal (ULN).

Virological Response (figure 1)

The median change from baseline in serum HBV DNA for these patients who received adefovir at study weeks 48, 144, 192, and 240 were -3.44, -3.69, -3.55, and -4.05 log_{10} copies/mL, respectively. 39% had serum HBV DNA < 1000 copies/mL at study week 240.
Biochemical Response (figure 2)

The median change from baseline in serum ALT concentrations for these patients who received adefovir at study weeks 48, 96, 144, 192, and 240 were -43, -18, -49.5, -41, and -50 IU/L, respectively; 66% had normalized (ULN) serum ALT concentrations at study week 240.

Serological Response (figure 3)

The proportion of patients with e-seroconversion increased steadily from baseline with the estimated Markov probability values of HBeAg seroconversion, 13%, 29%, 37%, 35%, and 30% for weeks 48, 96, 144, 192, and 240, respectively.

Fifteen patients had baseline and end of follow-up liver biopsies; improvements in necroinflammation and fibrosis were seen in 67% and 60% of these patients, respectively. Adefovir-associated substitutions, A181V or N236T, were detected in 16 of 38 (42%) patients in the setting of virologic failure (confirmed increase of ≥1 log10 HBV DNA copies/mL above nadir or never suppressed below 10^3 copies/mL) There were no serious adverse events related to ADV. In summary, the results of this long-term cohort study show that among HBeAg-positive patients, long term therapy with ADV achieves and maintains virological, biochemical, serological, and histological improvement. ADV was also well-tolerated.

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