INTRODUCTION

Upper gastrointestinal hemorrhage is the most common complication of peptic ulcers. In approximately 20% of patients, ulcer-related hemorrhage occurs without preceding symptoms (1). Bleeding persists or recurs in a subgroup of these patients with bleeding gastric ulcers. Eighty-four patients with peptic ulcers and 100 controls were studied between January 1998 and April 2000. We used polymerase chain reaction and endonuclease digestion to genotype for 4G/5G polymorphism in the promoter region of the PAI-1 gene and the Alu-repeat insertion/deletion (I/D) polymorphism in intron h of the t-PA gene. Various clinical features, including lesion site, bleeding event, recurrence of ulcer, and rebleeding, were assessed using a multiple logistic regression model. The genotype distributions of both the t-PA and PAI-1 genes did not differ between the patient and control groups. The occurrence of the I/D or D/D genotype of t-PA was significantly higher in cases of duodenal ulcer (adjusted OR=4.39, 95% CI=1.12-17.21). When a dominant effect (i.e., 4G/4G or 4G/5G versus 5G/5G) of the 4G allele was assumed, the PAI-1 4G/4G genotype was independently associated with rebleeding after hemostasis (adjusted OR=5.07, 95% CI=1.03-24.87). Our data suggest that t-PA gene polymorphism is associated with duodenal ulcers, and that the PAI-1 gene may be a risk factor leading to recurrent bleeding after initial hemostasis.

Key Words: Tissue Plasminogen Activator; Plasminogen Activator Inhibitor 1; Peptic Ulcer; Fibrinolysis; Hemostasis

Tissue Plasminogen Activator and Plasminogen Activator Inhibitor Type 1 Gene Polymorphism in Patients with Gastric Ulcer Complicated with Bleeding

Hong-Soo Kim, Kyu-Yoon Hwang*, Il-Kwon Chung, Sang-Heum Park, Moon-Ho Lee, Sun-Joo Kim, Sae-Yong Hong
Departments of Internal Medicine and *Preventive Medicine, Soonchunhyang University Chunan Hospital, Soonchunhyang University, Chonan, Korea

Received: 4 September 2002
Accepted: 1 November 2002

Address for correspondence
Sae-Yong Hong, M.D.
Department of Internal Medicine, Soonchunhyang University Chunan Hospital, 23-20 Bongmyung-dong, Chonan 330-100, Korea
Tel: +82-41-570-2121, Fax: +82-41-574-5762
E-mail: syhong@schch.co.kr

58

58
to provide local activation at the site of fibrin deposition.

Various methods have been used to evaluate systemic fibrino-
ytic activity, such as monitoring the euglobulin fibrinolyt-
ic activity, chromogenic bioassay, enzyme-linked immunosor-
ent assay, and radioimmunoassay for t-PA and PAI-1. How-
ever, there are several reasons why these methods do not pro-
vide an accurate measure of the patient’s physiologic fibrinolyt-
ic activity: (a) physiologic fibrinolysis does not occur in the
circulating blood but at the fibrin and/or thrombus site, so any
method that measures fibrinolytic activity in a sample of cir-
culating blood does not reflect fibrin-associated fibrinolysis;
(b) the release of t-PA from endothelial cells can be modified
by many factors such as exercise, venous occlusion, mental
stress, sympathetic stimulation, and prostaglandin (20); and
(c) variations in blood levels of PAI-1 are resulted from changes
in the rate of variations in blood levels of PAI-1 are resulted from changes
stress, sympathetic stimulation, and prostaglandin (20); and
by many factors such as exercise, venous occlusion, mental
stress, sympathetic stimulation, and prostaglandin (20); and
variational blood levels of PAI-1 are resulted from changes
in the promoter region of the PAI-1 gene is related to circulating
PAI-1 levels (21-24). In vitro experiments have shown that both alleles of the 4G/5G polymorphism contain a binding site
for a transcription activator, and that the 5G allele also
contains a binding site for a transcription repressor that partial-
ly overlaps with the activator binding site (21, 25). Therefore,
the 4G allele of the PAI-1 gene is associated with increased
basal gene transcription. The 4G/4G genotype of PAI-1 has been
shown to be associated with ischemic heart disease (24,
26) and myocardial infarction (MI) (21, 27).

Similarly, the insertion/deletion (I/D) genotype of Ala-repeat
insertion/deletion (I/D) polymorphism in the t-PA gene has been
shown to be associated with the plasma PAI-1 antigen in
patients with MI (28). Since t-PA and PAI-1 levels are posi-
tively correlated, the Ala-repeat I/D polymorphism could be a
marker of a functional mutation in the t-PA gene that reg-
ulates the interaction between t-PA and PAI-1. The Ala-repeat
insertion may also be closely linked to a mutation at or near
the t-PA gene that produces a functional effect, and an Alu-
repeat I/D event can alter mRNA stability and splicing (29).
Although Ala-repeat I/D polymorphism in the t-PA gene is not
associated with either t-PA plasma levels (28) or the basal
synthesis rate of t-PA in endothelial cells (30), Alu-repeat I/D
polymorphism in the t-PA gene may be a candidate marker
for thrombosis and hemostasis (31).

The major pathogenic mechanisms for peptic ulcer are acid,
HP, and NSAIDs (1). Of these factors, only NSAIDs appear
to be an important risk factor for the development of bleeding
(32-34). In order to avoid any influence of this factor in our
study, we excluded any patient who had a history of NSAID
within 3 months of the study.

Intramucosal proteolysis has been considered to be an aggres-
sive factor in the development of peptic ulcers, and it has been
shown that there is a significantly higher level of protease activ-
ity at the ulcer edge and from the antral mucosa of patients
with an active duodenal ulcer (15-17). HP can induce peptic
ulceration through direct production of several proteases capa-
bale of damaging the gastroduodenal mucosa (34, 35). It is well
known that plasmin, the end product of the fibrinolytic sys-
tem, converts latent collagenase to an active form. The main-
tenance of an adequate mucosal blood flow by adequate fib-
rinolysis is another essential protective factor (28, 29), and t-
PA may be a key enzyme in this process. Nonetheless, the role
of the fibrinolytic system on the development of peptic ulcers
and/or ulcer bleeding is controversial.

Taking these observations into consideration, we hypothe-
sized that the t-PA and PAI-1 genes are factors leading to
peptic ulcer and hemorrhage. In order to determine whether
our hypothesis was correct, we analyzed I/D type polymor-
phism in intron h of the t-PA gene and 4G/5G polymorphism
in the promoter of the PAI-1 gene in patients with gastric ul-
cers with or without ulcer bleeding.

MATERIALS AND METHODS

Study subjects

Between January 1998 and April 2000, diagnostic and/or
therapeutic endoscopy was performed in 84 patients with ac-
tive peptic ulcers. The control group comprised 100 subjects
who presented consecutively for multiphasic health check-up
at Soonchunhyang University Chunan Hospital. Individuals
with an NSAID-induced ulcer, underlying systemic disease,
or bleeding tendency were excluded from the study.

The study design was approved by the Ethics Committee
of Soonchunhyang University Chunan Hospital. Informed
consent was obtained from all subjects.

We used historical information and endoscopic examinations
to evaluate the lesion site for the presence of bleeding, recur-
rency, and HP infection. We performed several tests for HP
infection, including a rapid urease test (CLO test; Ballard,
Draper, Australia) that was observed for up to 24 hr, histological
examination of an endoscopic biopsy, 13C-labeled urea breath
test (UBT), and serologic examination for the presence of imm-
unoglobulin G antibody to HP (Radim, Pomezia, Italy). We
determined the presence of HP infection in patients when two
or more of the above tests produced positive results. Rebleed-
ing was defined as the persistence or redevelopment of overt
bleeding (hematemesis, melena, or hematochezia) or a reduc-
tion in hemoglobin of more than 1.5 g/dL within 5 days of
initial hemostasis.

Blood sampling and DNA isolation

Blood samples were obtained from the antecubital vein be-
tween 7 a.m. and 9 a.m. after a fast and a 20 min rest. For DNA
analysis, blood was collected in K-EDTA vacuum tubes, and stored at -70°C. DNA was isolated using a commercial genomic DNA purification kit (QIAamp; QIAGEN, Valencia, CA, U.S.A.). Serum was separated from whole blood and used for biochemical assays.

**Determination of genotypes**

Allele-specific polymerase chain reaction (PCR) was performed according to Falk's assay (52) with some modifications. The PAI-1 4G/5G polymorphism was determined for each subject using two 17-mer allele-specific primers: 5'-GTCTGGACACGTGGGG3' for insertion 5G allele and 5'-GCTGGACACGTGGGA3' for deletion 4G allele, in combination with the common downstream primer 5'-TCCGTAACAGGACACCTCA-3', located upstream of the polymorphic region in combination with the downstream primer. Each DNA amplification was performed in a total volume of 25 µL using 150-200 ng of genomic DNA. The reaction mixture contained 50 mM Tris-HCl buffer (pH 9.0), 1.5 mM MgCl2, 0.2 mM of each deoxyribonucleoside triphosphate, and 0.6 M of each primer, and 1 unit of DNA polymerase. The mixture was subjected to 35 155-sec-long cycles of 94°C for 1 min, 65°C for 35 sec, and 72°C for 1 min. The PCR products were separated by a fourth primer, 5'-AAGCITTTTACATGTGAAGCC-3', located upstream of the polymorphic region in combination with the downstream primer. Each DNA amplification was performed in a total volume of 25 µL using 150-200 ng of genomic DNA. The reaction mixture contained 50 mM Tris-HCl buffer (pH 9.0), 1.5 mM MgCl2, 0.2 mM of each deoxyribonucleoside triphosphate, and 0.6 M of each primer, except for the upstream control primers which were 0.12 µM and 1.0 U of DNA polymerase. The mixture was subjected to 35 155-sec-long cycles of 94°C for 35 sec, 65°C for 45 sec, and 72°C for 75 sec. The PCR products were separated by 2% agarose gel electrophoresis, stained with ethidium bromide, and viewed under UV light.

An I/D polymorphism resulting from the presence or absence of an Alu repeat in intron h of the t-PA gene was identified in all subjects. Briefly, 25-50 ng of each genomic DNA sample was amplified by PCR in 25-µL reaction volumes. Upstream (5'-TCCGTAACAGGACACCTCA-3') and downstream (5'-ACCGTGCTTCAGTCACTGGA-3') primers were chosen as previously described (31). Briefly, 50 ng of DNA was amplified in a 25 µL reaction containing 45 mM Tris-HCl (pH 8.0), 11 mM ammonium sulfate, 4.5 mM MgCl2, 6.7 mM 2-mercaptoethanol, 4.4 mM K-EDTA (pH 8.0), 1 mM each of dNTP, 100 µg/mL bovine serum albumin, 0.01% gelatin, 10 pmol of each primer, and 1 unit of Taq polymerase. The reaction was initially heated to 94°C for 5 min, which was followed by 30 3-min-long cycles of 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min. Amplified products (I allele=967 base pairs, D allele=655 base pairs) were resolved on 1.5% agarose gels and visualized by ethidium bromide fluorescence. Samples were classified according to three genotypes: I/I, D/D, and I/D. The classification was determined independently by two individuals who had no knowledge of the case or control status.

The PCR direct sequencing was carried out from randomly selected samples to confirm the accuracy of allele-specific oligonucleotide genotyping.

**Statistical analysis**

Differences in baseline characteristics among three groups were evaluated by analysis of variance (ANOVA) and χ2 tests for continuous and categorized variables, respectively. The observed numbers of each PAI-1 genotype and each t-PA genotype were compared with those expected for a population in Hardy-Weinberg equilibrium using a χ2 test. Associations of PAI-1 and t-PA genotypes with the presence of peptic ulcers were estimated using odds ratio (OR) and 95% confidence interval (CI) measures. Both dominant and recessive models were constructed to test the associations. Multiple logistic regression was applied to evaluate the independent association of the PAI-1 4G/5G polymorphism and t-PA I/D polymorphism with peptic ulcer, whilst controlling for possible confounding factors. Gene-gene interaction between PAI-1 and t-PA on peptic ulcer parameters was also assessed. Statistical analysis was performed using SPSS for Windows V 10.0 (SPSS Inc., Chicago, U.S.A.). P values less than 0.05 were considered to be statistically significant.

**RESULTS**

**Profile of patients**

The clinical profiles of the patients are given in Table 1. Genotypes for PAI-1 and t-PA were available in 84 patients with peptic ulcer and 100 and 98 of the controls, respectively. The mean (SD) age of the patients was 58.6 yr (15.1 yr), and 67 (79.8%) of them were male. The peptic ulcer was gastric in 56 cases and duodenal in 28 cases; its phase was categorized as initial in 64 cases and recurrent in 20 cases. Clinical bleeding was observed in 56 cases (66.7%). Of 32 patients subjected to the CLO test, 84.4% were positive. Fifty-six (66.7%) of the patients regularly drank alcohol, and 59 (70.2%) were cigarette smokers.

The clinical outcomes are presented in Table 2. Sixty patients required blood transfusion and 4 patients needed surgical operation for hemostasis. Rebleeding was observed in 9 patients. The mean (SD) duration of hospital treatment was 14.1 (8.8) days.

**Gene polymorphisms of t-PA and PAI-1**

The distribution of t-PA genotypes in 84 patients with a peptic ulcer was: 32 (38.1%) D/D, 30 (35.7%) I/D, and 22 (26.2%) I/I. This distribution was not significantly different from that of 98 control subjects (p=0.180): 25 (25.5%) D/D, 40 (40.8%) I/D, and 33 (33.7%) I/I (Table 3). No significant
The distribution of PAI-1 genotypes in the patients was: 26 (31.0%) 4G/4G, 42 (50.0%) 4G/5G, and 16 (19.1%) 5G/5G. This distribution was not significantly different from that of the 100 control subjects: 21 (21.0%) 4G/4G, 53 (53.0%) 4G/5G, and 26 (26.0%) 5G/5G ($p = 0.245$, Table 3).

### Table 3. Distributions of genetic polymorphisms of t-PA and PAI-1 in patients and controls

| Genetic polymorphisms | Patients n (%) | Controls n (%) | p-value |
|-----------------------|---------------|----------------|---------|
| t-PA                  |               |                |         |
| I                    | 22 (26.2)     | 33 (33.7)      | 0.180   |
| ID                   | 30 (35.7)     | 40 (40.8)      |         |
| DD                   | 32 (38.1)     | 25 (25.5)      |         |
| PAI-1                 |               |                | 0.245   |
| 4G/4G                | 26 (31.1)     | 21 (21.0)      |         |
| 4G/5G                | 42 (50.0)     | 53 (53.0)      |         |
| 5G/5G                | 16 (19.1)     | 26 (26.0)      |         |

### Table 4. Associations between gene polymorphisms and site of ulcer

| Gene | Model | Adjusted OR* | 95% CI | p value |
|------|-------|--------------|--------|---------|
| t-PA | Dominant stomach vs duodenum | 2.05 | 0.75-5.56 | 0.161 |
|      | Recessive stomach vs duodenum | 4.39 | 1.12-17.21 | 0.034 |
| PAI-1| Dominant stomach vs duodenum | 0.85 | 0.24-2.97 | 0.799 |
|      | Recessive stomach vs duodenum | 1.16 | 0.41-3.26 | 0.783 |

*adjusted for age, gender, alcohol consumption, and smoking.

### Table 5. Associations between gene polymorphisms and rebleeding

| Gene | Model | Adjusted OR* | 95% CI | p value |
|------|-------|--------------|--------|---------|
| t-PA | Dominant no vs rebleeding | 1.83 | 0.41-8.25 | 0.429 |
|      | Recessive no vs rebleeding | 3.95 | 0.40-38.66 | 0.237 |
| PAI-1| Dominant no vs rebleeding | 5.07 | 1.03-24.87 | 0.045 |
|      | Recessive no vs rebleeding | 0.97 | 0.20-4.58 | 0.966 |

*adjusted for age, gender, bleeding during endoscopy, ulcer site, alcohol consumption, and smoking.

### Relations of gene polymorphism with ulcer site and rebleeding

Using a multiple logistic regression model in which a recessive effect (I/I vs. I/D or D/D) of the I allele was assumed, the I/I genotype of t-PA was found to be related to the presence of peptic ulcers. The occurrence of the I/D or D/D genotypes of t-PA was significantly higher in duodenal ulcer patients (adjusted OR = 4.39, $p = 0.034$). However, PAI-1 was not related to ulcer site in both dominant and recessive models (Table 4).

When a dominant effect (4G/4G or 4G/5G versus 5G/5G) of the 4G allele was assumed, the PAI-1 4G/4G genotype was
significantly and independently related to rebleeding after initial hemostasis (adjusted OR = 5.07, p = 0.045). However, neither the recessive model of PAI-1 nor recessive or dominant model of t-PA was related to rebleeding (Table 5).

The interactions between PAI-1 and t-PA were calculated in both dominant and recessive models and evaluated for gene-gene interaction on ulcer sites and rebleeding as well as other clinical parameters in a further analysis. However, no significant interactions were observed (data not shown).

DISCUSSION

In the present study, the occurrence of the I/D or D/D genotypes of t-PA was higher in duodenal ulcers, and the PAI-1 4G/4G genotype was associated with rebleeding of a peptic ulcer. These results suggest that the t-PA and PAI-1 system plays a role in both ulcer formation and ulcer bleeding.

In our study, when age, gender, alcohol consumption, and smoking habits were controlled, there was an association between the I/D or D/D genotype of t-PA and the presence of a duodenal ulcer. This result suggests that the t-PA gene is an independent factor in the pathogenesis of duodenal ulcers. The so-called peptic ulcer disease encompasses both gastric and duodenal ulcers (1). Duodenal and gastric ulcers share many common features in terms of pathogenesis, diagnosis, and treatment, but several factors distinguish the two. Gastric ulcers tend to occur later in life than duodenal lesions. More than half of gastric ulcers occur in males, and they are less common than duodenal ulcers. As for duodenal ulcers, the majority of gastric ulcers can be attributed to either HP-or NSAID-induced mucosal damage. Gastric ulcers that occur in the prepyloric area or those associated with a duodenal ulcer are induced mucosal damage. Gastric ulcers that occur in the prepyloric area or those associated with a duodenal ulcer are induced mucosal damage, and many common features in terms of pathogenesis, diagnosis, and treatment, but several factors distinguish the two. Gastric ulcers tend to occur later in life than duodenal lesions. More than half of gastric ulcers occur in males, and they are less common than duodenal ulcers. As for duodenal ulcers, the majority of gastric ulcers can be attributed to either HP-or NSAID-induced mucosal damage. Gastric ulcers that occur in the prepyloric area or those associated with a duodenal ulcer are induced mucosal damage. Gastric ulcers that occur in the prepyloric area or those associated with a duodenal ulcer are induced mucosal damage.

Further studies are needed to clarify the property of the I/D polymorphism in the t-PA gene on peptic ulcer formation and leads to an accumulation of fibrin. The 4G allele and the 4G/4G genotype of PAI-1 have been reported to be associated with increased basal gene transcription, and consequently have been regarded as thrombogenic factors (21, 24, 26, 27). With this kept in mind, our results are suggestive of some other role of PAI-1 in the process of rebleeding, except the effect on fibrinolytic activity.

PAI-1 is synthesized by endothelial cells and hepatocytes, and is present mainly in platelets and plasma. But some PAI-1 is also present in the subendothelial extracellular matrix, where its function may be inhibition of local proteolysis. PAI-1 activity induces changes in remodeling of the vessel wall through activation of metalloproteinases, growth factors, and degradation of the extracellular matrix (26, 27). In addition to its antiprotease activity, PAI-1 participates in the cellular adhesion and migration processes (36). Taken the well-known function of PAI-1 to inhibit local proteolysis into consideration, our results suggest that disturbed vessel wall remodeling may be involved in the rebleeding of peptic ulcers.

In the normal physiological state, the activities of coagulation and fibrinolysis are balanced. However, increased activity of either coagulation or fibrinolysis can overwhelm the other, to the extent of producing a clinical problem. Therefore, increased fibrinolysis and/or decreased coagulation may be involved in the pathogenesis of ulcer hemorrhage. However, we believe that the role of t-PA and PAI-1 in peptic ulcer bleeding differs between current-bleeding and rebleeding situations. Furthermore, it seems likely that peptic ulcer rebleeding is due in large part to the properties of PAI-1, which disturbs remodeling of injured vessel after initial bleeding.

In conclusion, our data suggest that t-PA gene polymorphism may be associated with duodenal ulcers, and that the PAI-1 gene may be a risk factor leading to recurrent bleeding after initial hemostasis.

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