Bioequivalence of Two Intravenous Formulations of Antithrombin III: A Two-Way Crossover Study in Healthy Korean Subjects

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ABSTRACT

Background: Treatment with antithrombin (AT)-III is indicated for patients with sepsis or hereditary AT deficiency.

Objective: The purpose of this study was to compare the pharmacokinetic and pharmacodynamic characteristics of 2 AT-III formulations in healthy Korean volunteers to satisfy the regulatory requirements for bioequivalence for marketing purposes.

Methods: A single-center, single-dose, open-label, randomized, 2-period, 2-sequence crossover study was conducted in healthy Korean volunteers. Blood samples for the drug analysis were collected for up to 216 hours after drug administration. Participants received either the test or reference formulation of AT-III 100 U/kg IV for 20 minutes in the first period and the alternative formulation in the second period. Both the AT-III activity and antigen (Ag) were measured for the analysis of pharmacokinetic properties, and the prothrombin time and the activated partial thromboplastin time were assessed for the analysis of pharmacodynamic properties. Because AT-III is an endogenous compound, the analysis used data corrected from baseline values. The tolerability of the 2 formulations was also assessed based on physical examinations including vital sign measurements, laboratory tests, and 12-lead ECG.

Results: Of the 20 subjects enrolled (mean [SD] age, height, and weight, 25.3 [2.3] years, 175.3 [4.5] cm, and 67.4 [6.3] kg, respectively), 19 completed both treatment periods; 1 subject withdrew consent for personal reasons. The observed mean (SD) C_{max}, AUC_{last}, and $AUC_{0-\infty}$ of AT-III activity were, respectively, 279.24% (35.92), 14,364.10 (2325.25) %·h, and 17,526.38 (3150.81) %·h with the test formulation and 249.75% (31.96), 12,962.95 (1897.52) %·h, and 15,957.67 (3189.21) %·h with the reference formulation. The observed mean (SD) C_{max}, AUC_{last}, and $AUC_{0-\infty}$ of AT-III Ag were 62.58 (5.66) mg/dL, 3051.94 (401.87) mg/dL·h, and 3639.80 (726.01) mg/dL·h, respectively, with the test formulation and 58.63 (5.27) mg/dL, 2805.08 (272.38) mg/dL·h, and 3340.00 (428.46) mg/dL·h with the reference formulation. The geometric mean ratios (90% CI) of the log-transformed data for AT-III activity between the 2 formulations were 1.11494 (1.08994–1.14053) for C_{max}, 1.11305 (1.05435–1.17503) for AUC_{last}, and 1.11527 (1.03754–1.19889) for $AUC_{0-\infty}$; corresponding values for AT-III Ag were 1.08802 (1.06258–1.11405), 1.10905 (1.05804–1.16242), and 1.11460 (1.02058–1.21726). During the study period, 8 adverse events were reported, and all were transient, mild, and resolved completely during the treatment period.

Conclusion: The results of the present study showed that these 2 AT-III formulations met the regulatory criteria for pharmacokinetic bioequivalence with respect to AT-III activity and Ag in these healthy Korean subjects. ClinicalTrials.gov identifier: NCT00846274. (*Clin Ther*. 2013;35:1752–1761)

Key words: antithrombin III, bioequivalence, pharmacokinetic equivalence, pharmacokinetics.

INTRODUCTION

Antithrombin (AT)-III is a 58,000-Da, vitamin K–dependent glycoprotein that inhibits coagulation factors and is synthesized in the liver.[1,2] AT-III acts as a physiologic inhibitor of blood coagulation, and thus plays a key role in hemostasis.[3]

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Primarily AT-III treatment is indicated for sepsis or hereditary antithrombin deficiency.[4] In addition, it has the potential to have antiangiogenic, antitumor, and antiviral activities.[4–6] In the case of sepsis, the blood concentration of AT-III falls by 20% to 40% in septic patients, and the levels of AT-III correlate with disease severity and clinical outcomes.[7,8] The reduction of AT-III levels occurs for various reasons, as follows: decreased production of AT-III in the liver; inactivation of the enzyme elastase, which is increased during inflammation; and the loss of AT-III from the circulation into tissues through inflamed and leaking capillary blood vessels.[9] Patients with hereditary AT-III deficiency are at increased risk for venous thromboembolism, particularly in high-risk situations, such as surgery, trauma, and pregnancy. It has been reported that the risk for venous thromboembolism in these situations increases 10- to 50-fold compared with the general population.[10,11]

AT-III is used through the intravenous route for the management of acute thrombotic episodes and for prophylaxis of thrombotic episodes in patients. In healthy adults, the AT-III activity level ranges from 0.8 to 1.2 U/mL, with an average of 1 U/mL.[12] Therefore, the goal of treatment is to maintain the antithrombin activity at 80% to 120% of normal to prevent thromboembolic episodes.[4] The loading dose is based on targeting the 100% activity level in healthy patients, and is typically followed by subsequent doses to maintain the antithrombin activity between 80% and 120%, which is usually achieved with 60% of the loading dose.[4]

The most common and convenient laboratory method used for assessing AT-III is the detection of antithrombin activity.[13] Antigenic testing to measure AT-III levels directly has been used in a limited number of studies; this test is primarily used for distinguishing qualitative from quantitative results.[13] These antithrombin tests are performed after a thrombotic event has resolved and when a patient is suspected to have decreased AT-III levels clinically.

Even though it has been reported that the elimination half-life of AT-III is 56.8 to 68.0 hours,[4,14–16] little information is available concerning the pharmacokinetic characteristics of AT-III. Furthermore, the authors could not find any studies revealing the pharmacokinetic properties of AT-III in Asian populations, including Koreans.

Like the conventional formulation of AT-III, the newly developed AT-III is isolated from the pooled plasma in humans. Preclinical data revealed that the new formulation has physicochemical characteristics similar to those of the conventional AT-III formulation, but with greater purity (99% vs 98%) (Physicochemical analysis test for AT-III formulations [Study no, INPAC-06MS-203], Preclinical Data on Investigator's Brochure, SK Chemicals, 2008). Additionally, the new formulation was shown to have excellent viral safety against hepatitis A, B, and C; HIV; and parvovirus B19, and efficacy similar to that of conventional AT-III in rats (validation of virus removal and inactivation [Study no, 2/06.159], AT-III Efficacy test in experimental DIC model [Study no, M07002-3], Preclinical Data on Investigator's Brochure, SK Chemicals, 2008).

A literature search revealed that studies on the pharmacokinetic properties of AT-III, including bioequivalence evaluations, are lacking. Different from the conventional formulation of AT-III, recombinant AT-III was developed to treat patients with antithrombin deficiency.[4] In a comparison of the pharmacokinetic properties between recombinant AT-III and conventional AT-III, recombinant AT-III had greater clearance but a shorter half-life.[17]

The primary objectives of this study were to compare the pharmacokinetic characteristics between, and to determine the bioequivalence of, the newly developed and conventional formulations of AT-III in healthy Korean subjects for the purpose of registration approval of the test formulation in Korea.

SUBJECTS AND METHODS

Study Design

This single-center, single-dose, randomized, open-label, 2-sequence, 2-period, comparative crossover study was conducted at the Clinical Trial Center of Anam Hospital, Korea University College of Medicine, Seoul, Korea. The study protocol and informed-consent form were reviewed and approved by the institutional review board at Anam Hospital and by the Korea Food and Drug Administration (registration no. 693). The study was conducted in accordance with the principles of the Declaration of Helsinki, as outlined in the guideline for Good Clinical Practice.[18,19]

All data obtained from the study were monitored by the sponsor. Even though the present study was an open-label study, the observer was neutral in relation to the participants and the investigational drugs. The purpose and procedures of the study were explained to the participants in detail before the study, and all subjects provided written, informed consent before participating in the study. Participants were supplied with written information that included the study purpose, methods,