INTRODUCTION:
Osteonecrosis of the femoral head (ONFH) is one of the most serious complications induced by corticosteroid therapy. In patients with ONFH, collapse of femoral head often occurs and causes severe hip pain and impaired hip joint function. Despite the widely spread use of corticosteroids for treating various diseases and a known association between prevalence of ONFH and daily dose of corticosteroids, pathomechanism for the development of ONFH has not been identified.

Since hepatic cytochrome P4503A (CYP3A) is a predominant enzyme responsible for metabolizing the corticosteroids and its activities varies more than 10-folds, low hepatic CYP3A activity leads to a remarkable increase of corticosteroid levels and its effect. We have previously reported that hepatic CYP3A levels are significantly lower in patients with steroid-induced ONFH than that in control patients and patients with alcohol-related ONFH and that hepatic CYP3A activity inversely correlated with the incidence of osteonecrosis and extent of the necrotic area caused by the same dose of corticosteroids in a rabbit model, suggesting possible prevention of the steroid-induced osteonecrosis by adjusting steroid dose based on the level of individual hepatic CYP3A activity.

To examine hepatic CYP3A activity, the measuring clearance of administered midazolam (MDZ) is a reliable method, as shown by the significant correlations between the clearance of midazolam and hepatic CYP3A levels measured by biopsy and the clearance of other CYP3A-specific substrates. However, the method needs multiple blood samplings about half a day for each object. The study was designed to develop the simple, safe and less-invasive methods for measuring the level of hepatic CYP3A activity, which is applicable to prevent the occurrence of steroid-induced osteonecrosis. This method could be used simply, safely and less-invasively for patients prior to corticosteroid therapy, and the adjusting dose of corticosteroids, tailor-made medicine, depending on the CYP3A activity of the individual patient could avoid the occurrence of steroid-induced osteonecrosis.

MATERIALS AND METHODS:

Substudy
Thirty seven healthy, male (n=20) and female (n=17) volunteers with a mean ± standard deviation (SD) age of 26.7±4.4 years (range: 19–37 years), a mean height of 166.4±8.2 cm (range: 150–183 cm), a mean weight of 59.5±11.7 kg (range: 41–83 kg) and a mean body mass index (BMI) of 21.3±2.6 kg/m² (range: 16.6–26.3 kg/m²) were included. All participants underwent routine laboratory tests that included haematology, blood chemistry, and serologies. Particular attention was given to renal and hepatic functions.

All medication use was prohibited during one week preceding the trial and throughout the trial period. None of the participants received any drug that would possibly activate or inhibit the microsomal hepatic enzymes during two weeks preceding the study administration in accordance with the protocol. This protocol was approved by our Institutional Committee on Human Research. Each volunteer gave written informed consent to participate in this study.

Procedures
Each volunteer had a single dose oral administration. The study drug, 50µg/kg of very small quantity of MDZ (injectable solution given as an oral solution: 20 mg/2 ml), was administered orally with 100 ml of room temperature water around 8 a.m., after fasting for at least 10 h. Water was given ad libitum from 2 h after dosing. Standardized meals were served 4 h after administration (lunch). Subjects abstained from consuming beverages and food containing alcohol or methylxanthines (chocolate, coffee, tea, cola), grapefruit and orange (juice and whole fruit) from 24 h before the study drug was administered and during the whole study period.

Blood samples (1 ml at each time) were collected in tubes containing lithium heparin before MDZ administration and at 15, 30, 45, 60, and 90 min and 2, 3, 4, 6, 9 and 12 h postdrug administration, and immediately centrifuged to obtain plasma, which was stored at −70°C until analysis. Concentrations of total MDZ and its principal metabolite, 1′-hydroxymidazolam (1′-OH MDZ), were measured by one of the authors (Y.O.), who was blinded to the identity of the samples by use of validated liquid chromatography-tandem mass spectrometry (LC-MS) method as reported previously. Clearance of midazolam was calculated as dose divided by area under the plasma concentration–time curve with computer software (Sigma Plot version 10.0; Systat Software, San Jose, CA, USA).

In addition, the subjects participated in the Observer’s Assessment of Alertness/Distraction Scale (OAAS/S) to evaluate the effectiveness of MDZ objectively, which classifies 5 scales on reactivity (5 for normal, 3 for drowsiness and 1 for no reactivity).

Statistical analysis
Repeated linear regressions between the ratio of 1′-OH MDZ: MDZ plasma concentrations and the clearance of MDZ and between OAAS scale and the clearance of MDZ, i.e. at each sampling time, were carried out in order to find the best correlation. Correlations were assessed by the Spearman coefficient with SAS statistical software (9.1; SAS Institute, Cary, NC, USA).

RESULTS:

Distribution profile of MDZ clearance
The distribution of MDZ clearance is summarized in Figure 1. These results show a large inter-individual variability in MDZ clearances (12.0 ± 4.5 (mL · kg⁻¹ · min⁻¹), range: 5.2–23.4). For all subjects, the peak concentration was observed within 30 minutes post-dosing. Mean plasma concentrations of MDZ and 1′-OH MDZ are plotted in Figure 2. MDZ concentrations declined exponentially.

Figure 1: Distribution of MDZ clearance

Figure 2: Mean plasma concentrations of MDZ and 1′-OH MDZ

Single-point correlations
The best correlations between MDZ clearance and the ratio of 1′-OH MDZ: MDZ plasma concentrations measured at each experimental time intervals were observed at 4 h (R² = 0.83) post-dosing (Figure 3), and better correlations were found at 3 h with a strong correlation (R² = 0.81). The good correlations between MDZ clearance and OAAS/S scale were found at 15 minutes (p = 0.04).

DISCUSSION:
Despite the young healthy volunteers, a single MDZ plasma measurement taken at 4 h post-oral administration may represent an accurate marker of CYP3A activity, and the evaluation of reactivities at 15 min post-oral administration can be objective marker of CYP3A activity.

This single-point blood sampling method could be used simply, safely and less-invasively for patients prior to corticosteroid therapy, and the adjusting dose of corticosteroids, tailor-made medicine, depending on the CYP3A activity of the individual patient could avoid the occurrence of steroid-induced osteonecrosis.

REFERENCES: