INTRODUCTION

Edoxaban is a therapeutic direct inhibitor of coagulation Factor Xa (FXa). Following oral administration as Edoxaban tosylate, peak concentrations are observed within 1-2 hours. Edoxaban undergoes biotransformation, producing many metabolites, some of them keeping Anti-FXa activity (M4-M6-M8), and the most abundant (M4) is formed through hydrolysis.

AIM

The aim of this work is to perform a comparative study for the measurement of Edoxaban and its active metabolites using commercial or modified anti-FXa bio-assis and LC/MS:MS on plasmas from healthy volunteers who received Edoxaban and to evaluate the impact of metabolites on the global anti-Xa activity measured.

RESULTS

Following Edoxaban intake, rapid absorption occurred, resulting in peak plasma concentrations at 1 to 2 hours in compliance with former reports, followed by a decline phase. Concentrations are about 10% higher with bio-assis than with LC/MS: MS, especially between 10 and 24 hours, which correlates with the increase of metabolites’ concentration. Anti-FXa assays measure the global activity of all forms (Edoxaban and M4-M6-M8 metabolites), whereas LC/MS:MS measures only Edoxaban or Edoxaban + M4 (Figure 1A). Plasma Edoxaban is the predominant species (88% to 96%), the most abundant active metabolite is M4 (3% to 9%), followed by M6 (1% to 2.5%) and M8 (0.1% to 2.2%). The amount of total active metabolites in plasma is approximately 10% corresponding to the difference of measurement between bio-assis and LC/MS: MS (Figure 1B).

The kinetics method showed lower global anti-FXa activity by comparison with the 2-stage method. Measurement of the metabolites with both bio-assis and influence of the FXa used for the assay evaluated.

CONCLUSIONS

Anti-FXa bio-assis are the most appropriate to measure the global anti-Xa activity of Edoxaban and of all its active metabolites (M4-M6-M8). Although metabolite M6 has a higher activity in the one stage assay, M6 represents less than 2.5% of the global anti-FXa activity. Thus, measurement differences between the 2 assays is negligible. Bio-assis demonstrate an excellent correlation with LC/MS:MS. They measure the global anti-Xa activity of Edoxaban and of all its active metabolites (M4-M6-M8), and they can be used for assessing low and very high concentrations.

METHOD

Anti-FXa assays:

BIOPHEN™ Heparin LRT method is a kinetics chromogenic assay based on the inhibition of a constant amount and in excess of bovine FXa by Edoxaban and active metabolites. The residual FXa hydrolyses a specific chromogenic substrate (Sxa-11) releasing paranitroaniline (pNA). The amount of pNA released (measured by absorbance at 405 nm) is inversely proportional to the concentration of anti-Xa product.

The modified BIOPHEN™ Heparin LRT method is a kinetics chromogenic assay using human FXa instead of bovine FXa.

BIOPHEN™ DiXal is a 2-stage chromogenic method based on the inhibition by Edoxaban and its active metabolites of a constant and in excess quantity of human FXa. The residual Factor Xa hydrolyses the FXa-specific chromogenic substrate, releasing paranitroaniline (pNA). The amount of pNA released (measured by absorbance at 405 nm) is inversely proportional to the concentration of anti-Xa product.

Low (0 to 120 ng/mL) and high (0 to 500 ng/mL) calibration ranges are used on CS series, for measuring Edoxaban and its metabolites for their Anti-FXa activity

Plasma Samples:

Citrated plasma samples used for comparison study (Bioassays vs LC/MS:MS), are obtained from healthy volunteers who received Edoxaban (60 mg/Day, tested at 0,5; 1; 2; 10 and 24 hours) or from treated patients, and stored at ~70 °C until use. Normal plasmas are spiked with Edoxaban and metabolites (M4, M6 and M8) (kindly provided by Dalichi) for the bioassays comparison studies.

REFERENCES


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