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Bio-Assays for the measurement of Anti-FXa activity of Edoxaban and its metabolites in plasma

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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-assays 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.

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.

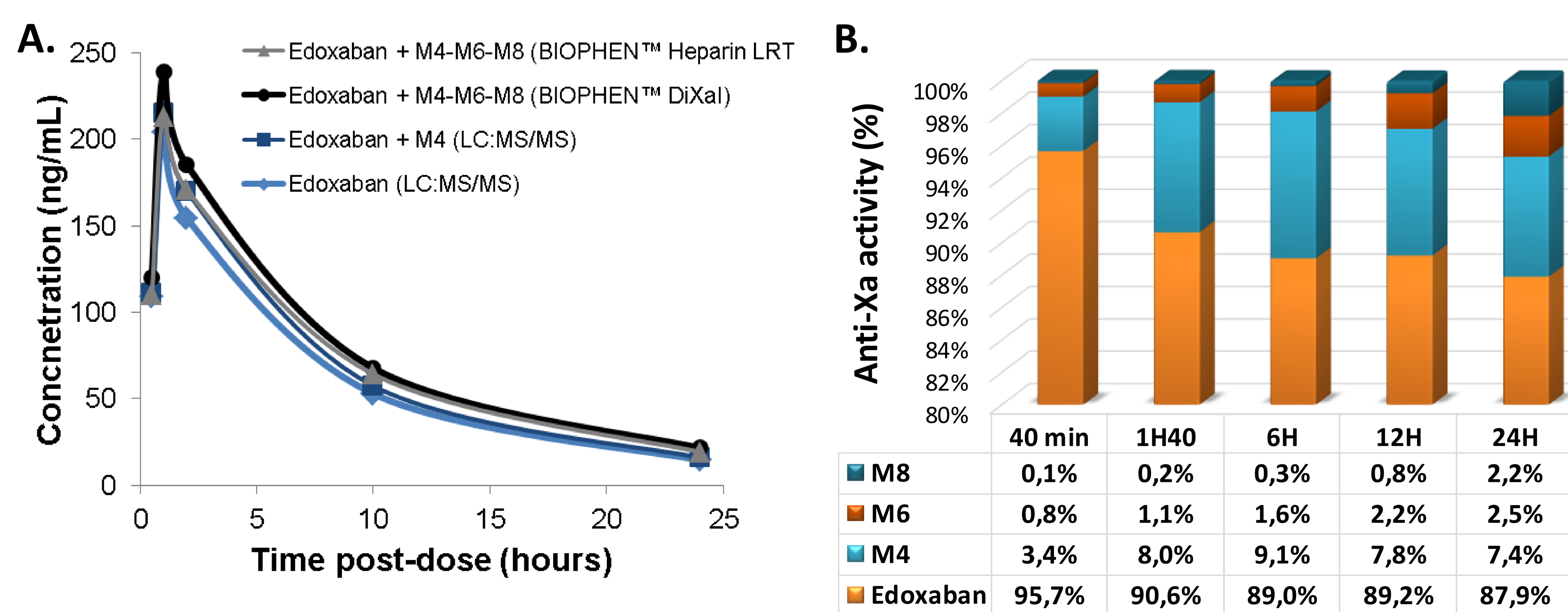
Low (0 to 120 ng/mL) and high (0 to 500 ng/mL) calibration ranges are used on CS series, for measuring Edoxaban and its metabolites for their Anti-FXa activity

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 Daïichi) for the bioassays comparison studies.

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-assays 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-assays and LC/MS: MS (Figure 1B).



	40 min	1H40	6H	12H	24H
M8	0,1%	0,2%	0,3%	0,8%	2,2%
M6	0,8%	1,1%	1,6%	2,2%	2,5%
M4	3,4%	8,0%	9,1%	7,8%	7,4%
Edoxaban	95,7%	90,6%	89,0%	89,2%	87,9%

Figure 1: (A) Edoxaban and active metabolites measurement with Bioassays and Edoxaban or Edoxaban + M4 metabolite measurement with LC:MS/MS in plasmas samples from healthy volunteers (collected at different times after intake). Following Edoxaban administration, rapid absorption occurred, resulting in peak plasma concentrations at 1 to 2 hours in compliance with former reports, followed by a decline phase. (B) Adapted from Bathala et al., 2012. Plasma concentrations of Edoxaban and active metabolites after a single oral dose of 60 mg of [^{14}C]Edoxaban.

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

Similar measurements are observed with both bio-assays, for M4 and M8 metabolites, but one stage method showed higher activity for metabolite M6 (Figure 2A) and this does not seem to be due to FXa source (Figure 2B). But the M6 anti-FXa activity is higher when the kinetics method is modified using human FXa for the assay (Figure 2C).

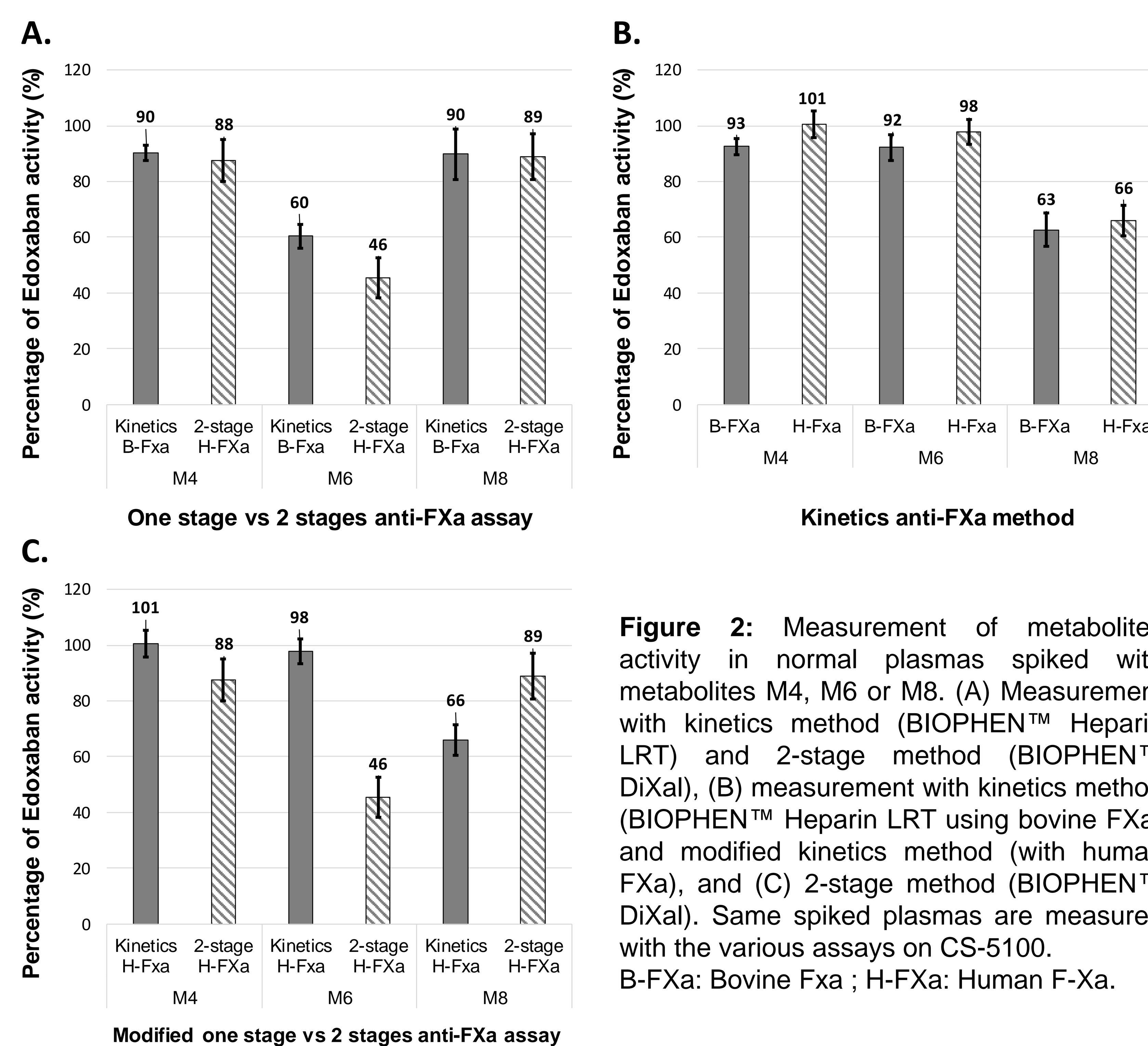


Figure 2: Measurement of metabolites activity in normal plasmas spiked with metabolites M4, M6 or M8. (A) Measurement with kinetics method (BIOPHEN™ Heparin LRT) and 2-stage method (BIOPHEN™ DiXal), (B) measurement with kinetics method (BIOPHEN™ Heparin LRT using bovine FXa) and modified kinetics method (with human FXa), and (C) 2-stage method (BIOPHEN™ DiXal). Same spiked plasmas are measured with the various assays on CS-5100.
B-FXa: Bovine Fxa ; H-FXa: Human F-Xa.

The concentration of metabolite M6 is maximal at 24 hours but the impact is very low on the global anti-FXa activity (less than 2.5%). The metabolite M4 is the most important metabolite in terms of concentration and the measurement of this metabolite is similar with both commercial bio-assays

CONCLUSIONS

Anti-FXa bio-assays 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-assays 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.

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