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Pharmacokinetic study based on atmospheric pressure MALDI-IT-TOF imaging mass microscope: A case of octreotide in mouse target tissues

ASMS 2017 ThP 287

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Introduction

Application of imaging mass spectrometry in drug pharmacokinetics remains challenging due to its weak quantitative capability. Herein, an imaging mass microscope (iMScope *TRIO*), equipped with an optical microscope, an atmospheric pressure ion-source chamber for matrix-assisted laser desorption/ionization (AP-MALDI) and a hybrid quadrupole ion trap time-of-flight (QIT-TOF) analyzer, was first validated and applied to visualize drug disposition *in vivo*. The distribution and elimination rate of the therapeutic peptide octreotide, a long-acting analogue of the natural hormone somatostatin, was first calculated based on the data determined by iMScope *TRIO* system combining a novel relative exposure strategy. Microspotted pixel-to-pixel quantitative iMScope *TRIO* provided a relative amount of octreotide presented in a thin stomach/intestinal section while preserving its original spatial distribution. The images of dosed mouse stomach clearly demonstrated the transport process of octreotide from the mucosal layer to the muscle side. More importantly, octreotide was found to eliminate from the intestines rapidly, the absorption peak time (T_{max}) appeared at 40 min and the half-life time ($t_{1/2}$) was calculated as 37.7 min according to the elimination curves. Comparisons to the LC-MS/MS data adequately indicated that the quantification approach and methodology based on the iMScope *TRIO* was reliable and practicable for drug pharmacokinetic study.

Methods and Materials

After intragastrical administration of octreotide at a dose of 50 mg/kg, the mice were sacrificed at 10, 20, 40, 60, 120, 240 and 360 min and mouse stomach and intestinal sections with 10 μm thickness were sliced, which then was coated by optimal matrix of α-CHCA (Sigma-Aldrich) via sublimation (iMLayer, Shimadzu, Kyoto, Japan) plus airbrushing manner. The spatial distribution and elimination of octreotide was evaluated by imaging octreotide and internal standard (IS) lanreotide in mouse stomach and intestine via iMScope *TRIO* (Shimadzu, Kyoto Japan). Absolute quantification analysis of octreotide was conducted by homogenization and separation of the remaining dosed and control gastrointestinal tissues via conventional LC-MS/MS (LCMS-8050, Shimadzu, Kyoto, Japan).

Figure 1 iMScope *TRIO* imaging mass microscope

Overlaid optical and MS images

Best-in-class 5 μm spatial resolution

Structural analysis by highly accurate MSn analysis

6 pixel per second, high-speed analysis

Ionization time: 50 ms, mass range:500-1000, MS mode

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Result

Selection of a matrix and its coating manner

Compared with DHB and SA , CHCA were chosen due to the average ion signal intensity of the octreotide. Two-step matrix application (sublimation plus airbrushing) was confirmed to be a suitable matrix coating manner according to ionization efficiency. Lanreotide, a structural analogue of octreotide, was used as the internal standard due to the similarity in their molecular weight and ionization characteristics.

The laser in the iMScope *TRIO* system was a diode-pumped 355 nm Nd:YAG laser and operated under the following parameters: frequency, 500 Hz; laser intensity, 25.0; pulse width, 5 ns, and the irradiated tissue surface with 50 shots for each pixel. The parameters of IT-TOF MS were set as follows: ion polarity, positive; mass range, 950 to1200; sample voltage,3.5 kV; detector voltage,1.90 kV.

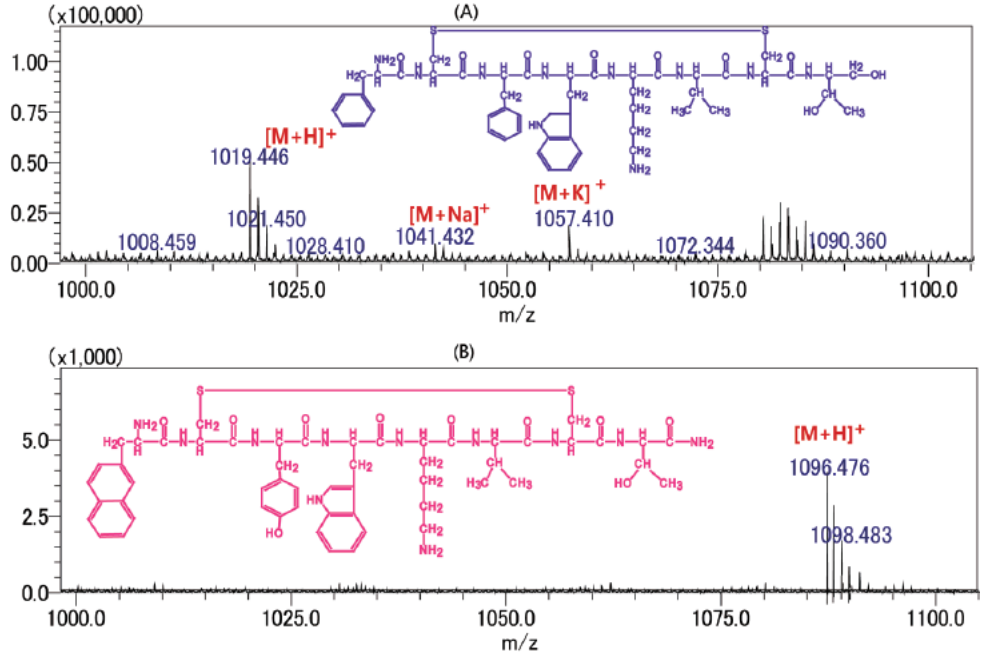

Figure 2 The structures and mass spectrum of octreotide and lanreotide (internal standard). A: octreotide; B: lanreotide.

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Construction of calibration curves of octreotide in drug free mouse stomach section

Figure 3 shows (a) optical image of the mouse tissue acquired by microscope via magnifying for 20X, (b) calibration curves of octreotide in mouse intestinal section, (c) imaging MS analysis of octreotide at 5 concentration levels (c1: 1 pmol, c2: 2 pmol, c3: 5 pmol, c4: 10 pmol, c5: 20 pmol) mixed with 10 pmol lanreotide which was used for the calibration of intensities of octreotide at 1, 2, 5, 10 and 20 pmol, respectively (c6 ~ c10).)

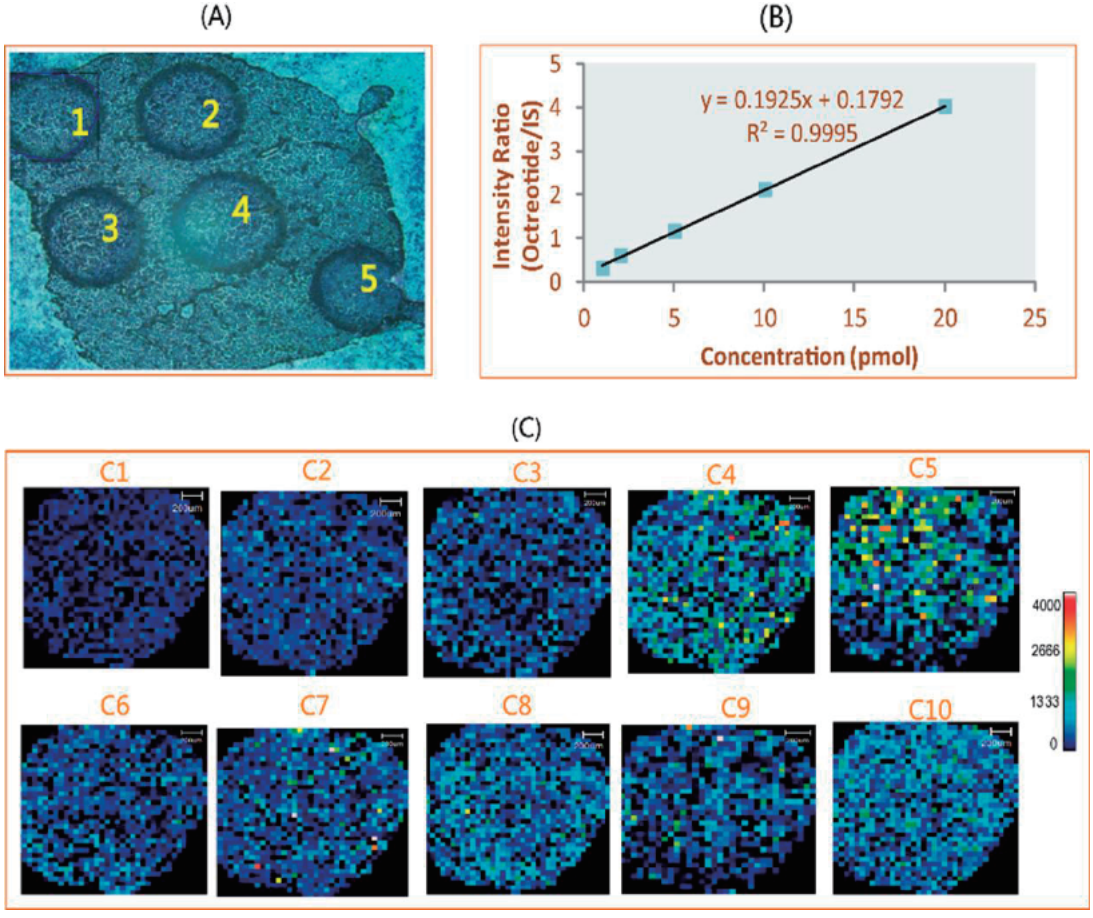

Figure 3 Calibration curves of octreotide constructed by plotting the average intensity ratios (octreotide/lanreotide) against the concentrations in mouse stomach section determined based on LDI-QIT-TOF-IMS (The scale bar was 50 μm).

Figure 3 shows (a) optical image of the mouse tissue acquired by microscope via magnifying for 20X, (b) calibration curves of octreotide in mouse intestinal section, (c) imaging MS analysis of octreotide at 5 concentration levels (c1: 1 pmol, c2: 2 pmol, c3: 5 pmol, c4: 10 pmol, c5: 20 pmol) mixed with 10 pmol lanreotide which was used for the calibration of intensities of octreotide at 1, 2, 5, 10 and 20 pmol, respectively (c6 ~ c10).)

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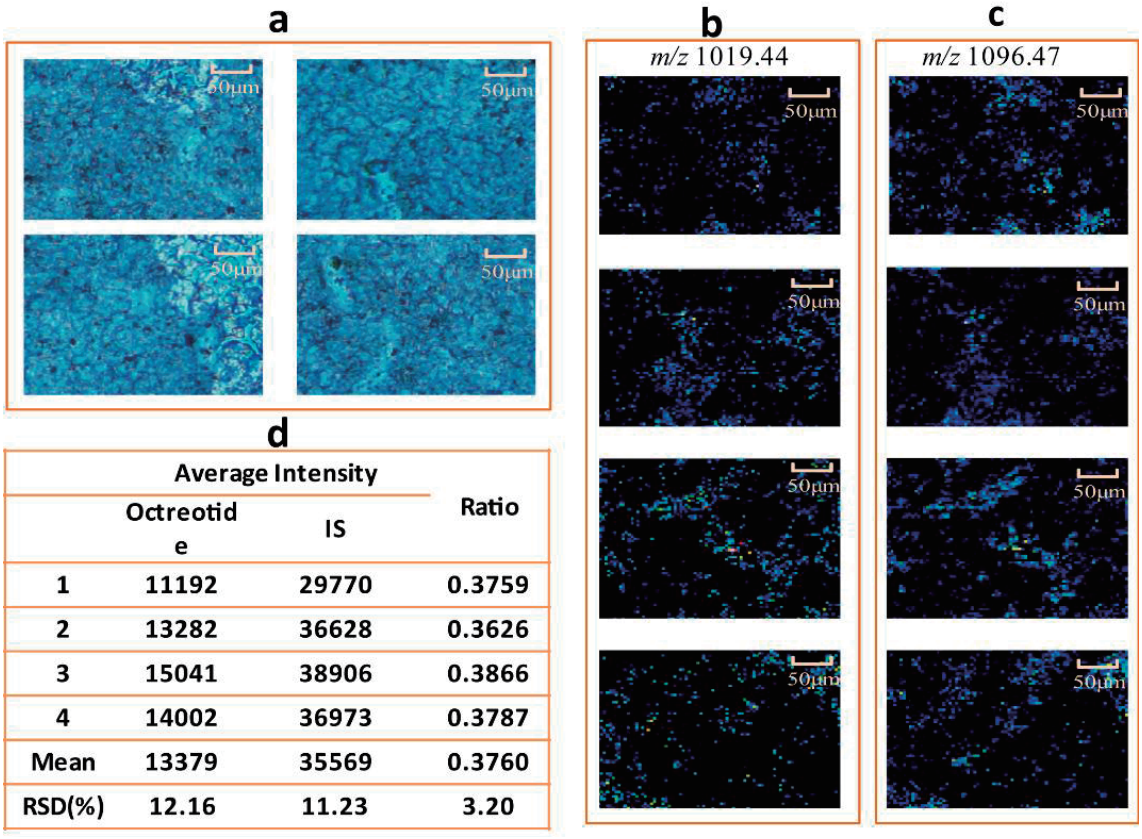
The verification of the method precision in drug free stomach section

Figure 4 displyas (a) optical image of mouse stomach section spiked with the standard solution of octreotide acquired by the CCD camera (magnification, X20) which embedded in the iMScope system; (b) imaging MS analysis of octreotide at m/z 1019.44; (c) imaging MS analysis of lanreotide at m/z 1096.47; (d) precision data analysis for octreotide



d

	Average Intensity		Ratio
	Octreotide	IS	
1	11192	29770	0.3759
2	13282	36628	0.3626
3	15041	38906	0.3866
4	14002	36973	0.3787
Mean	13379	35569	0.3760
RSD(%)	12.16	11.23	3.20

Figure 4 The average intensity ratio of octreotide and IS in four sections of blank stomach tissue covered by the same concentration of the analytes determined based on AP-MALDI-QIT-TOF-iMScope (The scale bar was 50 μm).

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The distribution and elimination of octreotide in mouse intestinal tract

Figure 5 shows the distribution of octreotide at 20 min (A), 40 min (B), 60 min (C), 120 min (D), 240 min (E) and 360 min (F) post dose (A1, B1~F1: magnified view of the mouse intestinal; A2,B2~F2: imaging MS analysis of octreotide; A3, B3~F3: imaging MS analysis of lanreotide for the calibration of intensities of octreotide at 20, 40, 60, 120, 240 and 360 min in mouse intestinal, respectively). (G) the time-concentration curve of octreotide in mouse intestinal.

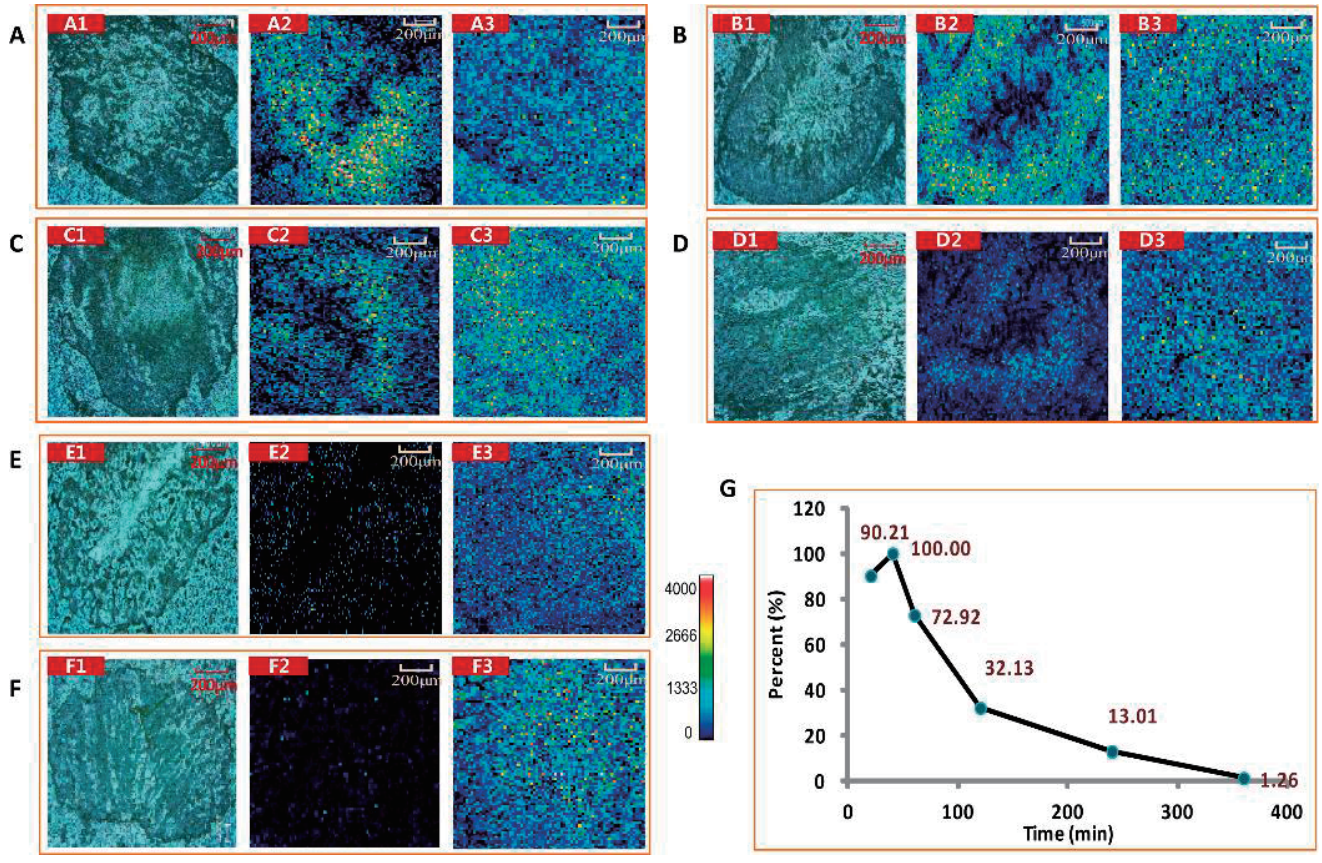

Figure 5 The distribution and elimination of octreotide in mouse intestinal after intragastrical administration of octreotide at a dose of 50 mg/kg. (The scale bar was 200 μm).

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The spatial distribution and elimination of octreotide in mouse stomach

Figure 6 shows the spatial distribution of octreotide at 10 min (A), 20 min (B), 40 min (C), 60 min (D) and 120 min (E) post dose (A1, B1~E1: magnified optical image of the mouse stomach; A2, B2~E2: imaging MS analysis of octreotide).

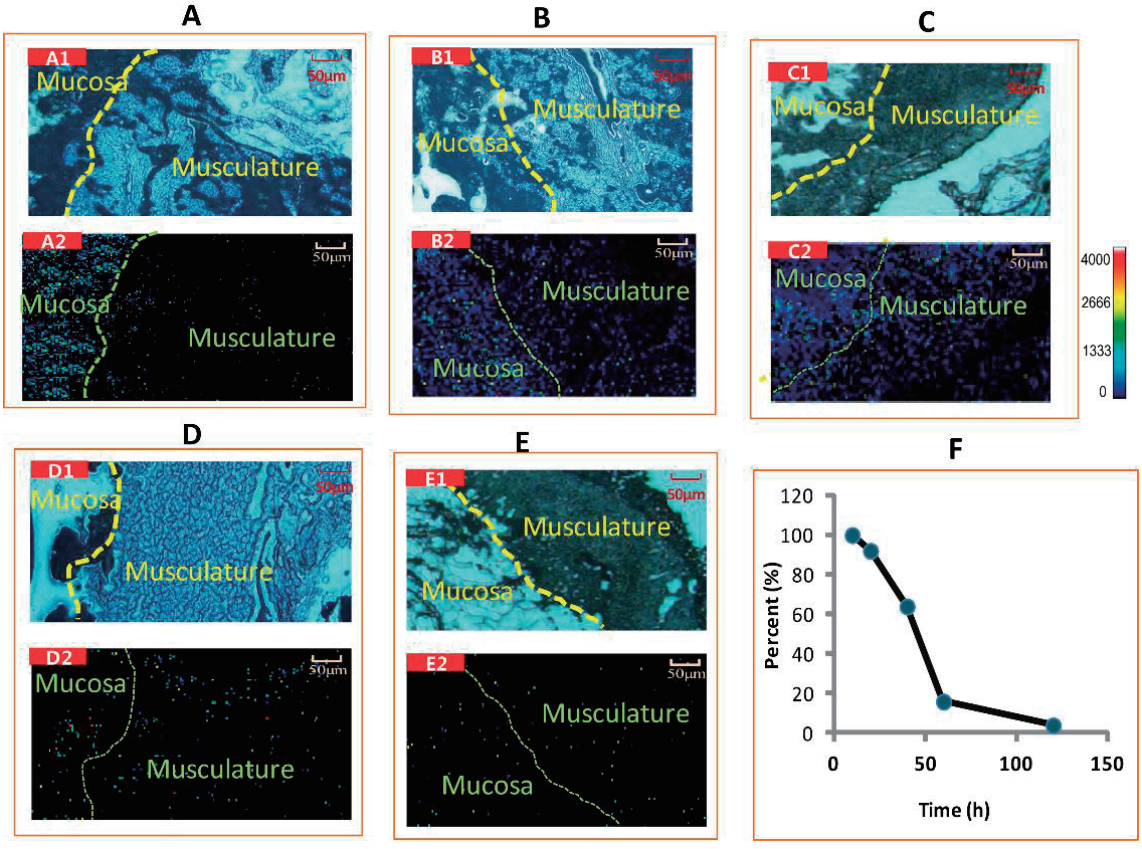

Figure 6 The spatial distribution and elimination of octreotide in mouse stomach after intragastrical administration of octreotide at a dose of 50 mg/kg. (The scale bar was 50 μm).

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Conclusions

The results showed that the absorption peak time of octreotide in mouse stomach and intestinal appeared at 10 min and 40 min, respectively. The half-life time in stomach and intestinal was calculated as 28.0 min and 37.7 min, respectively, according to the changing trend of peak intensity ratio (octreotide/IS). Comparisons to the LC-MS/MS data adequately indicated that the quantification approach and methodology based on the imaging mass microscope was reliable and practicable for drug pharmacokinetics.

Disclaimer: Shimadzu LCMS-8050 and iMScope *TRIO* are intended for Research Use Only (RUO). Not for use in diagnostic procedures.

First Edition: June, 2017

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