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PO-CON1476E

Characterization of metabolites in microsomal metabolism of aconitine by high-performance liquid chromatography/quadrupole ion trap/time-of-flight mass spectrometry

ASMS 2014 WP 739

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Introduction

Aconitine (AC) is a bioactive alkaloid from plants of the genus Aconitum, some of which have been widely used as medicinal herbs for thousands of years. AC is also well known for its high toxicity that induces severe arrhythmias leading to death. Although numerous studies have raised on its pharmacology and toxicity, data on the identification metabolites of AC in liver microsomes are limited. The study of metabolic pathways is very important for efficacy of therapy and evaluation of toxicity for those with narrow therapy window.
The aim of our work was to obtain the metabolic pathways of AC by the human liver microsomes.

Methods and Materials

Sample Preparation

The typical reaction mixture incubation contained 10 μ mol/L aconitine and was preincubated at 37 °C for 3 min. Reactions were initiated by adding 50 μL of NADPH (20 mmol/L), then incubated at 37 °C in a waterbath shaker for 60 min. The reactions were terminated by adding 3-volume of ice-cold acetonitrile, then vortexed and centrifuged to remove precipitated protein.

Instrument	: LCMS-IT-TOF (Shimadzu Corporation, Japan); UFLCXR system (Shimadzu Corporation, Japan);
Column	: Shim-pack XR-ODS II (2.0 mmI.D. x 75 mmL.,2.2 μm)
Mobile phase	: A: water (0.1% formic acid+5 mmol ammonium formate), B: acetonitrile
Gradient program	: 30%B (0-4 min)-80%B (8 min)-80%B (8-11 min)-30%B (11.01-17 min)
Flow rate	: 0.3 mL/min

Results

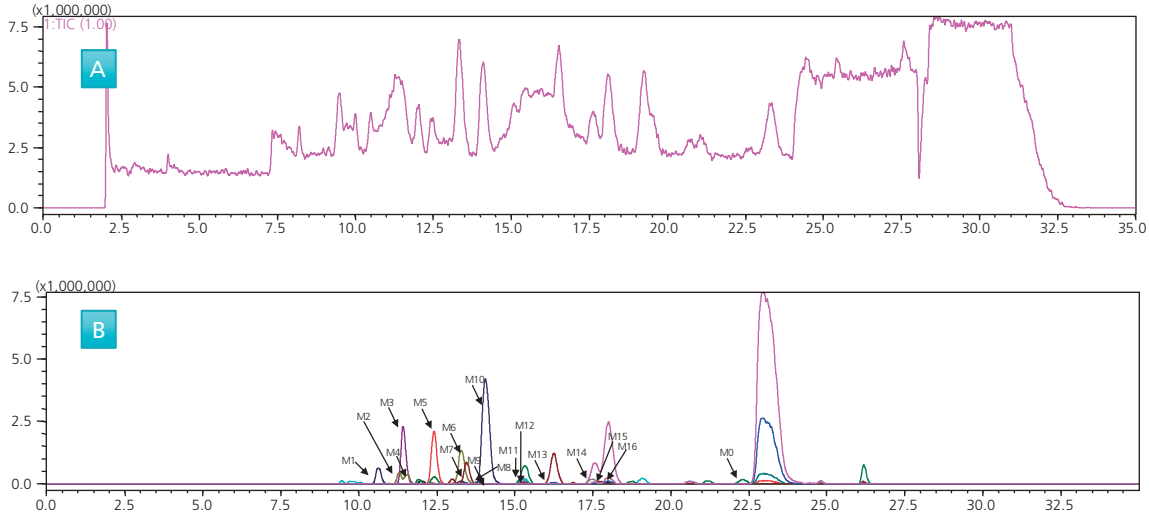

Fig.1 TIC chromatogram (A) and mass chromatograms of the metabolites of AC in the microsomal incubation mixture of human (B)

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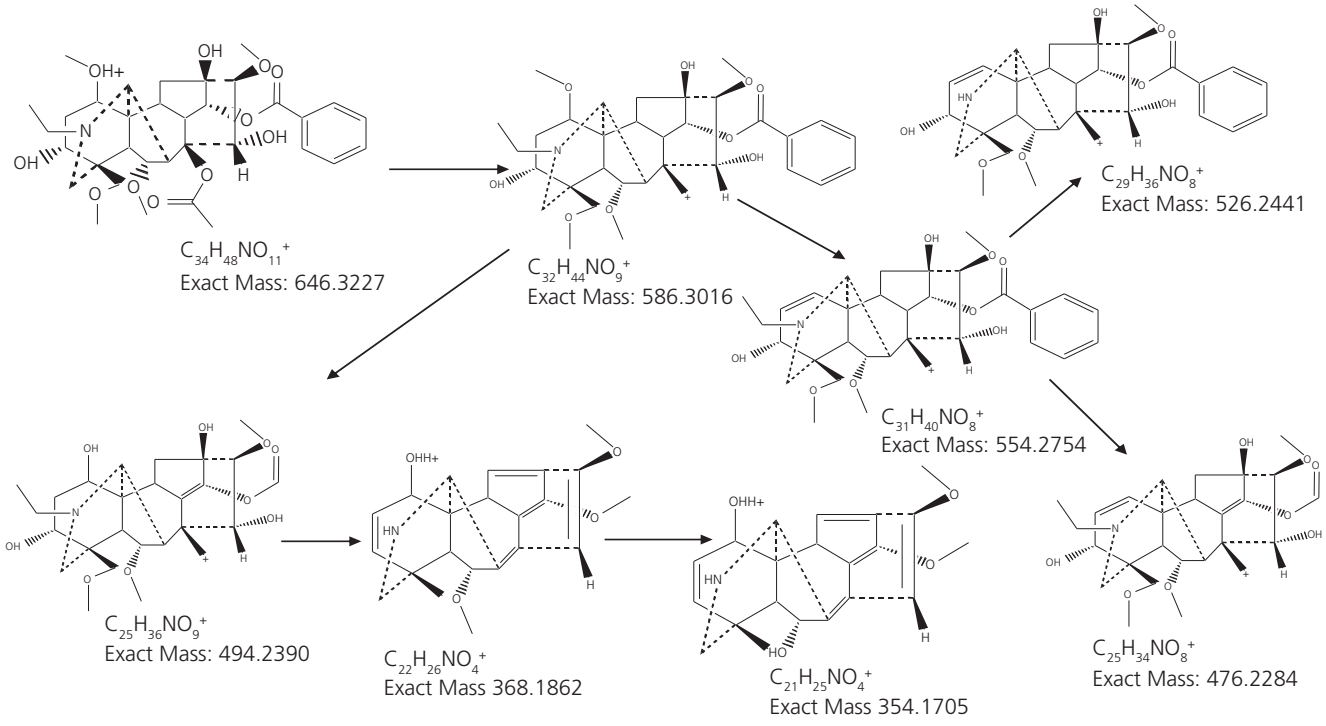

Fig. 2 Proposed fragmentation pathway of AC

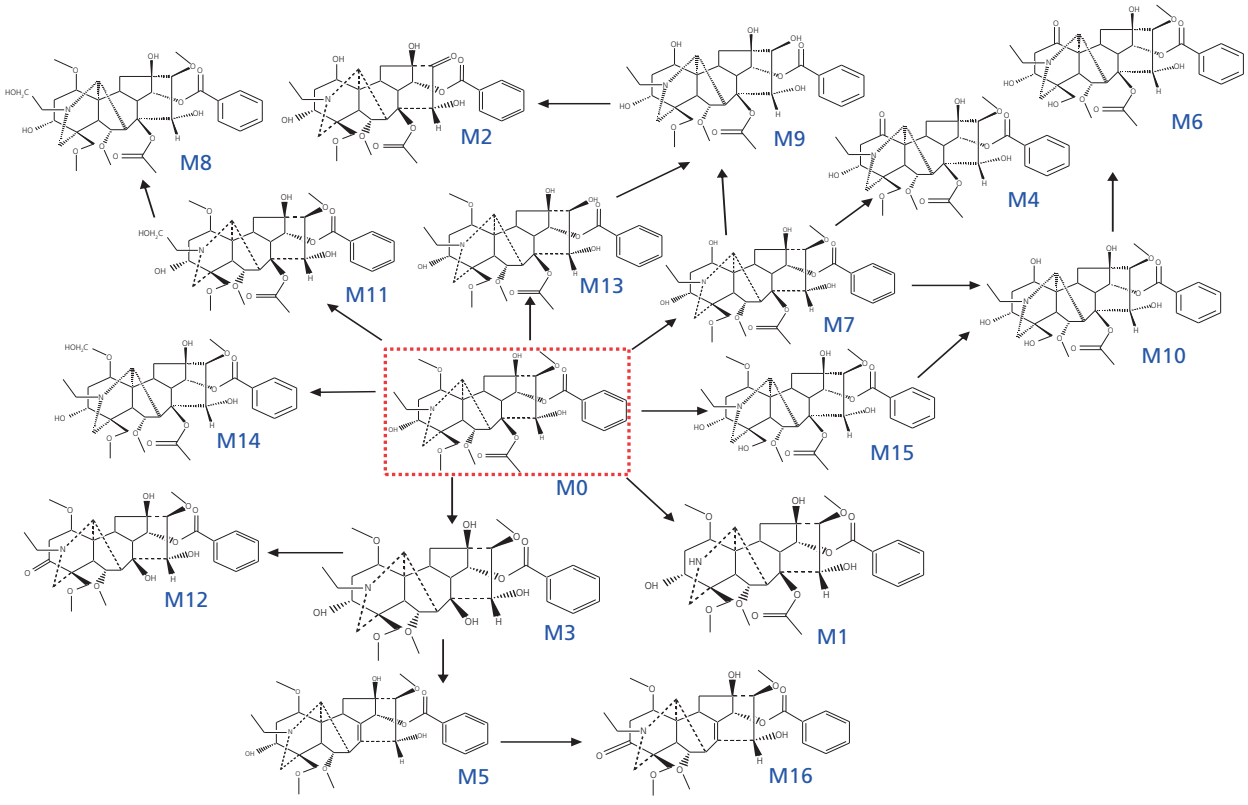

Fig. 3 Proposed metabolic profile of AC in the human liver microsomes

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Table1 Mass data for characterization of metabolites in of AC in the microsomal incubation mixture of human

No.	RT (min)	Meas.MW (*m/z*)	Pred.MW (*m/z*)	mDa error	ppm error	MS^2 data	Formula	Biotransformation
M0	22.3	646.3230	646.3222	0.8	1.26	586.3000, 554.2752, 526.2785, 494.2536, 476.2431, 404.2432, 368.1847, 354.1687	C34H47NO11	Parent
M1	10.5	618.2922	618.2909	1.3	2.10	558.2710, 498.2469, 480.2378, 436.2093, 354.1725	C32H43NO11	deethylation
M2	11.2	616.2754	616.2752	0.2	0.26	556.2510, 554.2335, 494.2106, 478.2321, 434.1908, 402.1682	C32H41NO11	bidemethylation+ dehydrogenation
M3	11.3	604.3140	604.3116	2.4	3.94	554.2744, 522.2398, 434.1898	C32H45NO10	deacetylation
M4	11.8	630.2930	630.2909	2.1	3.35	570.2686, 552.2576, 510.2457, 492.2381	C33H43NO11	demethylation+ dehydrogenation
M5	12.2	586.3005	586.3011	0.6	0.96	568.2938, 554.2705, 522.2537, 466.2168, 434.1922	C32H43NO9	deacetylation+ dehydration
M6	13.3	616.2769	616.2752	2.3	3.68	584.2477, 524.2316, 434.1941	C32H41NO11	bidemethylation+ dehydrogenation
M7	13.5	632.3035	632.3065	3.0	4.81	572.2866, 512.2638, 494.2468, 480.2283, 462.2214, 290.2236, 354.1652, 340.1871	C33H45NO11	demethylation
M8	13.7	648.3016	648.3015	0.1	0.23	588.2702, 570.2654, 528.2566, 510.2434, 406.2161	C33H45NO12	oxidation+ demethylation
M9	13.8	618.2935	618.2909	3.0	4.88	558.2714, 494.2109, 476.2400, 340.1548	C32H43NO11	bidemethylation
M10	14.1	618.2890	618.2909	1.5	2.43	558.2722, 494.2127, 476.2009, 354.1635	C32H43NO11	bidemethylation
M11	15.0	662.3179	662.3171	0.8	1.21	602.2964, 570.2654, 542.2750, 510.2434, 420.2416	C34H47NO12	oxidation
M12	15.1	602.2948	602.2960	1.6	2.66	584.2533, 524.2249, 510.2179, 406.1582	C32H43NO10	deacetylation+ dehydrogenation
M13	16.0	632.3054	632.3065	1.1	1.80	572.2853, 512.2661, 480.2368, 476.2445, 436.2082, 368.1812	C33H45NO11	demethylation
M14	17.3	662.3209	662.3171	3.8	5.74	602.2947, 570.2654, 542.2766, 510.2434, 478.2187	C34H47NO12	oxidation
M15	17.6	632.3068	632.3065	0.3	0.42	586.2973, 526.2738, 508.2273, 494.2490	C33H45NO11	demethylation
M16	17.9	584.2826	584.2854	2.8	4.82	552.2669, 492.2111, 460.2063	C32H41NO9	deacetylation+dehydration+ dehydrogenation

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Conclusions

In this study, totaling 16 metabolites were found and characterized in the humam liver microsomes incubation mixture, including *O*-demethylation, oxidation, bidemethylation, dehydrogenation, *N*-deethylation, deacetylation, dehydration and besides M1, M3, M4, M9, M13 and M15, all the left ten of them were first identified and reported. Collectively, these data provide a foundation for the clinical use of AC and contributes to a wider understanding of xenobiotic metabolism and toxicity evaluation.

First Edition: June, 2014

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