Inhibition of two NADPH-independent enzymatic activities: Aldehyde oxidase and Xanthine oxidase inhibition

Didier Bressac, Rachel Dechaume, Aurélie Péraire, Emmanuel Hardillier, Christelle Gondran, Eric Lecocq, Olivier Lacombe

ADME / PK Department - INVENTIVA - 50 rue de Dijon - 21121 Daix, France

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INTRODUCTION

Aldehyde oxidase (AO) and xanthine oxidase (XO) are referred as "molybdenum hydrolases". They are two NADPH-independent cytosolic enzymes which are involved in the metabolism of an extensive range of drugs, especially in the oxidation and reduction of aldehydes or N-heterocyclic compounds. As the importance of these both enzymes is growing in the metabolism of new drugs, the assessment of potential drug-drug interactions (DDIs) with them is more and more studied. The study describes the inhibition of the specific metabolism pathway of Phthalazine and Pterin, probe substrates of AO and XO conducting to the formation of Phthalazone and Isoxanthopterin, respectively, in the human liver cytosol. Raloxifen and Allopurinol were used as inhibitors of reference.

METHOD

Sample incubation:

Phthalazine, AO probe substrate, is incubated at 5 µM with human liver cytosol (0.5 mg/mL) in absence and presence of Raloxifen (several concentrations) as specific inhibitor for 15 and 30 minutes. The depletion of the Phthalazone formation was measured by LC-MS/MS.

Pterin, XO probe substrate, is incubated at 50 µM with human liver cytosol (0.5 mg/mL) in absence and presence of Allopurinol (several concentrations) as specific inhibitor for 60 and 120 minutes. The depletion of the Isoxanthopterin formation was measured by LC-MS/MS.

Analytical method:

The samples are injected into a LC-MS/MS system with the following conditions:

Equipment:

Instrument	Type	Manufacturer
Mass spectrometer	API 4000 QTRAP	MDS SCIEX, APPLIED BIOSYSTEM
LC system	PROMINENCE	SHIMADZU

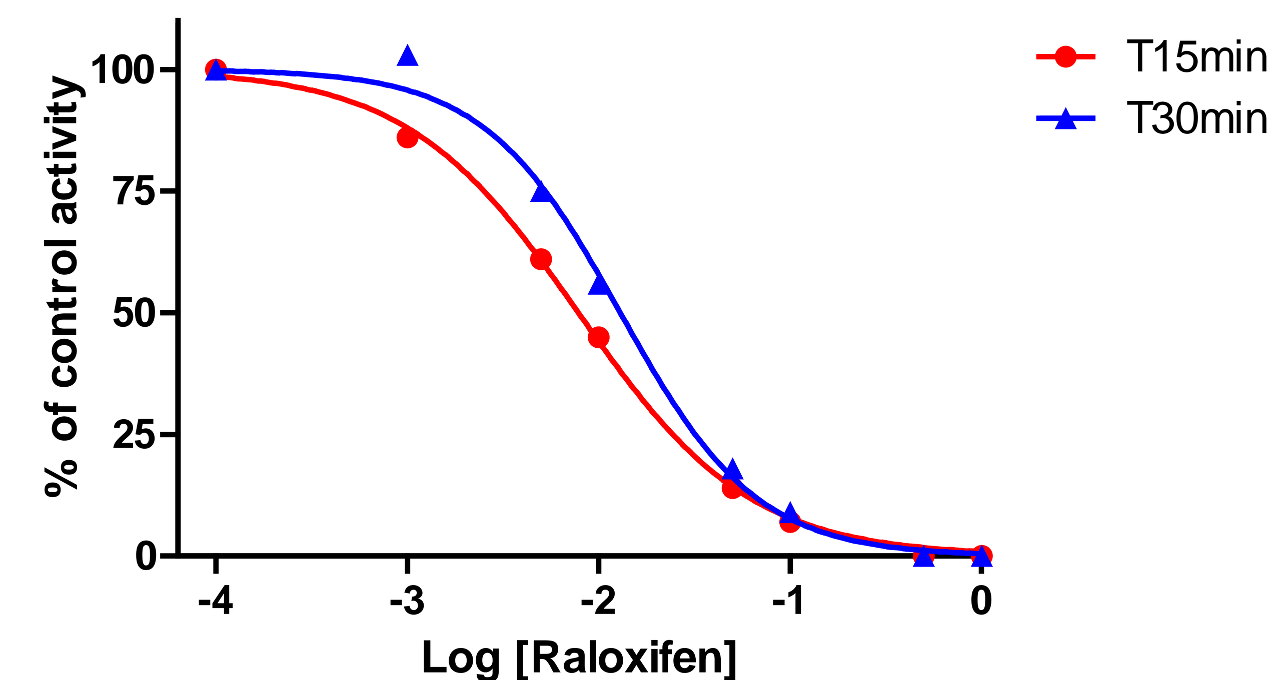
Chromatographic conditions:

Column	Atlantis dC18 4.6 x 150 mm, 3 µm (Waters)
Oven Temperature	45°C
Injection volume	2 µL

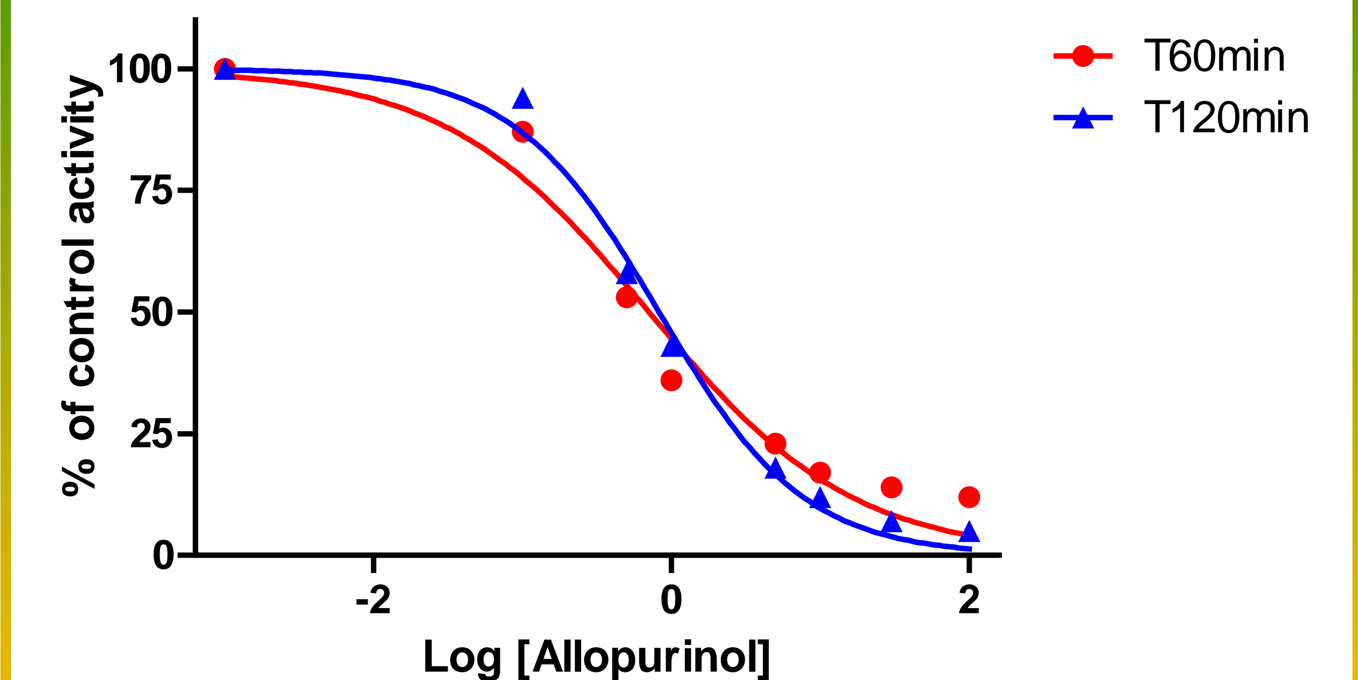
AO activity inhibition				XO activity inhibition			
Gradient				Gradient			
Time (min)	% Mobile Phase A water + 0.1% formic acid	% Mobile Phase B acetonitrile + 0.1% formic acid	Flow (mL/min)	Time (min)	% Mobile Phase A water + 0.1% formic acid	% Mobile Phase B acetonitrile + 0.1% formic acid	Flow (mL/min)
0	80	20	1	0	90	10	1
3.5	5	95		3.5	10	90	
4.8	5	95		4	10	90	
5	80	20		4.5	90	10	
7	80	20		6.5	90	10	
MS transition		147.1 → 90.1		MS transition		180.1 → 163.1	

Phthalazine and Pterin metabolic pathways

RESULTS



Incubation time (minutes)	LogIC50	IC50 (nM)	r2
15	-2.107	7.8	0.999
30	-1.888	12.9	0.995



Incubation time (minutes)	LogIC50	IC50 (nM)	r2
60	-0.1517	0.7	0.968
120	-0.0840	0.8	0.991

DISCUSSION

The inhibition of both aldehyde and xanthine oxidases activities were clearly demonstrated in these conditions by using the combination substrate/inhibitor, Phthalazine/Raloxifen and Pterin/Allopurinol respectively.

Phthalazone formation was inhibited by Raloxifen with an IC50 ~ 8-13 nM, and Pterin formation was inhibited by Allopurinol with an IC50 ~ 0.7-0.8 µM, confirming that Raloxifen and Allopurinol are potent inhibitors of the aldehyde and xanthine oxidase activity in these conditions, respectively.

These incubation conditions and the analytical methods allow a robust protocol for the assessment of a potential drug-drug interaction of drug candidates with aldehyde or/and xanthine oxidase, with Raloxifen and Allopurinol as positive controls.

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