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Molecular mechanism of an adverse drug–drug interaction of allopurinol and furosemide in gout treatment

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ABSTRACT

Gout patients receiving a combination of allopurinol and furosemide require higher allopurinol doses to achieve the target serum urate (SU) of <6 mg/dl (Stamp et al., 2012) [1]. Our study aimed to identify the molecular basis for this observation. We used a fluorimetric assay to determine the impact of furosemide and oxypurinol (the active metabolite of allopurinol) on xanthine oxidase (XO) activity. Immunoblot analysis quantified expression of XO and AMP-kinase (AMPK) in drug-treated human liver (HepG2) and primary kidney (HRCE) cells. *In silico* analysis identified miR-448 as a potential XO-regulator, whose expression level in HepG2 cells was examined by qPCR.

Fluorimetric experiments revealed no direct interactions between XO and furosemide, nor did the combination of oxypurinol/furosemide alter the XO inhibition profile of oxypurinol. In HepG2 cells, we found a significant decrease in XO protein expression following oxypurinol treatment, which was abolished after co-incubation with furosemide. Probenecid alone or in combination with furosemide reduced XO protein expression significantly. qPCR analysis of miR-448 in HepG2 cells mirrored the drug-dependent changes in XO protein expression. In addition, oxypurinol and the combination of oxypurinol/furosemide significantly down-regulated AMPK protein expression in HRCE cells.

In conclusion, we show for the first time that besides the established effects of allopurinol on the purine synthetic pathway the efficiency of allopurinol treatment of gout patients is based on two further complementary mechanisms, the direct inhibition of XO activity by the allopurinol metabolite oxypurinol and a down-regulation of XO protein expression. The latter is compromised by addition of furosemide and might explain why patients receiving furosemide therapy require higher allopurinol doses. miR-448 was identified as a potential drug-dependent XO regulator. Finally, down-regulation of AMPK protein expression in HRCE cells by administration of oxypurinol/furosemide reveals a possible new mechanism of renal drug-induced hyperuricemia.

1. Introduction

The xanthine oxidase (XO) inhibitor allopurinol, or rather its pharmacologically active metabolite oxypurinol [2], is the most common clinical treatment for abnormally high serum urate (SU) levels in gout patients [3,4]. Frequently, patients with gout also suffer from co-morbidities such as cardiac or kidney problems that require additional drug treatment with diuretics, particularly furosemide. Belonging to the class of loop diuretics, furosemide primarily acts by inhibiting NKCC2, the luminal $Na^+/K^+/2Cl^-$ symporter in the thick ascending limb of the loop of Henle. Studies have shown that the combination of furosemide and oxypurinol decreases the urinary excretion of uric acid and oxypurinol [5], and initially it was speculated that this interaction might render the hypouricemic effect of allopurinol more potent. A more recent clinical report by Stamp and co-workers [1] showed that patients receiving allopurinol and furosemide indeed exhibited increased plasma oxypurinol levels as well as increased SU. Compared to patients receiving a similar allopurinol dose alone, however, they needed higher allopurinol doses to achieve the target SU of <6 mg/dl. This observation indicates that the hypouricemic effect of allopurinol is in fact attenuated by the addition of furosemide. Increased SU after co-administration of furosemide is generally thought to be due to the plasma volume reduction or secondary enhancement of sodium re-absorption that is coupled to urate. However, the molecular mechanisms underlying this drug interaction are not yet understood.

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Oxypurinol works by competitively inhibiting the XO enzyme (Fig. 1A), but it is unknown whether furosemide exhibits a direct effect on XO as well or if it hampers the binding of oxypurinol to XO. The latter hypothesis could explain the observed adverse effect of the combination of furosemide and allopurinol. Even if direct interactions may be excluded, there are diverse regulatory pathways within the cell that could be subject to changes by the combination of furosemide and oxypurinol: (1) different expression of XO, (2) changes in levels of regulatory proteins or miRNAs, (3) changes in urate/drug transporter expression or function.

The latter has already partly been addressed. Renal secretion of diuretics such as furosemide or thiazides is crucial for their pharmacologic effect. However, this mechanisms of diuretic secretion has been recognised to cause high SU or hyperuricemia (SU > 7 mg/dl) [6,7]. We and others have shown that organic anion transporters such as OAT1 and OAT3 [8], OAT4 ([8–10]), NPT4 [11] or MRP4 [12] are involved in the renal secretion of diuretics such as furosemide (Fig. 1B). Since all of these transporters are also uric acid transporters, the concomitance of diuretics and urate alters normal excretion patterns: Diuretics can either inhibit the luminal secretion of uric acid (in case of unidirectional transporters such as NPT4 or MRP4) or the excretion of the diuretic can simultaneously facilitate the uptake of uric acid from the luminal side (OAT1–4). Both mechanisms lead to hyperuricemia.

Recently, studies have shown that AMP-kinase (AMPK) is involved in renal uric acid transport in avian renal proximal tubules [13]. Under stress conditions AMPK is activated, resulting in a decrease of uric acid secretion via MRP4, which is the only renal uric acid secreting transporter in birds. This illustrates that AMPK can be pivotal in the regulation of transporters that are involved in the clearance of urate and furosemide. Assuming a similar mechanism exists in humans, furosemide treatment could alter urate transport via activation of AMPK. Whether AMPK is affected by furosemide in human proximal renal tubule cells is currently unknown.

The aim of this study was to elucidate these drug interactions on a molecular level. Firstly, we studied direct interactions between oxypurinol and furosemide (alone and in combination) on purified XO enzyme in a cell-free assay. We then explored the effects of these drugs on expression levels of XO in cultured human liver cells (HepG2) by immunoblot. Moreover, we identified miR-448 as a potential XO-regulator and analysed its expression levels in drug-treated HepG2 cells. We also analysed AMPK expression in drug-treated primary human renal cortical epithelial (HRCE) cells

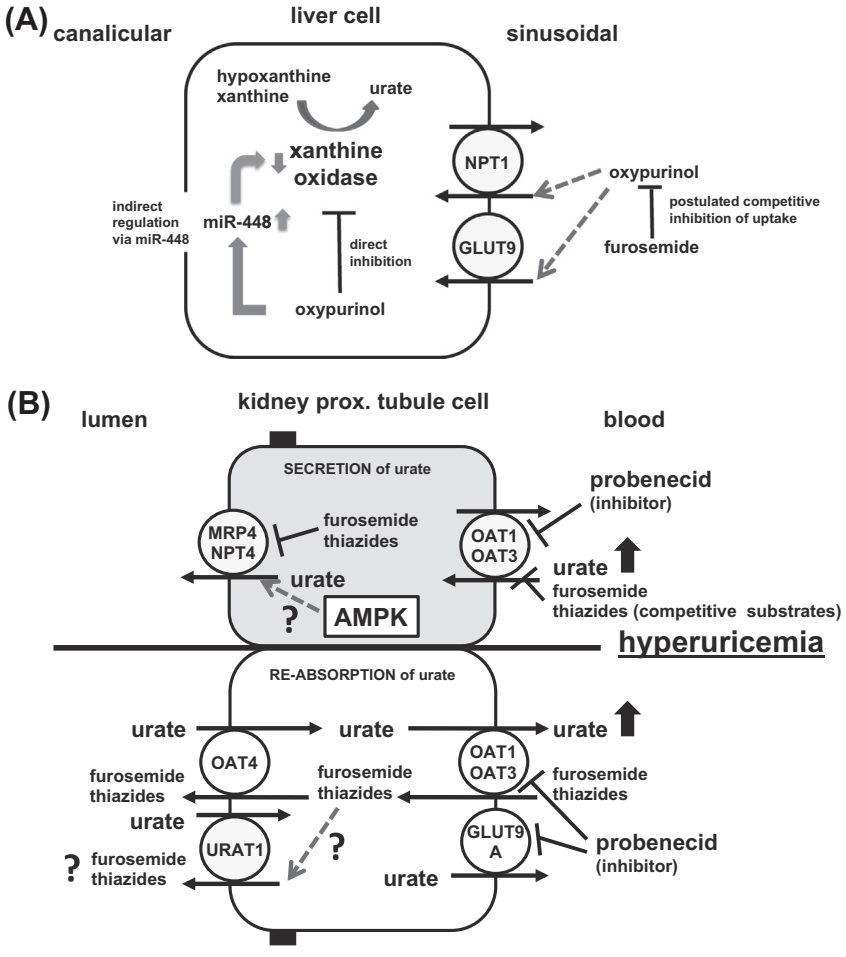

Fig. 1. Cell models for the effect of furosemide/probenecid on urate synthesis in the liver or urate transport in the kidney are shown. (A) Oxypurinol is transported into liver cells by proteins such as NPT1 or GLUT9. In the cytosol, it directly inhibits xanthine oxidase (XO) and consequently uric acid synthesis. We have identified miR-448 as potential post-transcriptional regulator of XO, which is regulated by drugs such as oxypurinol. (B) In the proximal tubule of the kidney, diuretics such as furosemide or thiazides have to be secreted from the blood into the urine to take effect. On the basolateral (blood) side, this secretion process is facilitated by organic anion transporters, namely OAT1 and OAT3. On the luminal side, furosemide and thiazides are released into the urine via MRP4 or NPT4 as well as OAT4 and URAT1. All these transporters are also urate re-absorbing (OAT4 and URAT1) or secreting (MRP4 and NPT4) transporters. Hyperuricemia (high SU) can be caused by the active secretion of diuretics (furosemide or thiazides) in exchange for urate, thus increasing the re-absorption of urate. It can also result from an inhibition of urate secretion by diuretics. Probenecid increases excretion of urate via inhibition of urate transporters on the blood and/or luminal side. In addition to these direct effects of diuretics on transporters as competitive substrates it has been reported that AMP-kinase can regulate urate transporters and consequently secretion or re-absorption of urate.