Analytica Chimica Acta 756 (2012) 60–72

Contents lists available at SciVerse ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Metabolic studies of the *Amaryllidaceous* alkaloids galantamine and lycorine based on electrochemical simulation in addition to *in vivo* and *in vitro* models

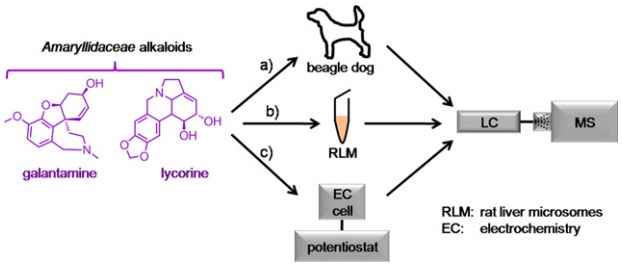
Sandra Jahn [a], Bettina Seiwert [b], Sascha Kretzing [b], Getu Abraham [b], Ralf Regenthal [c], Uwe Karst [a,*]

[a] *University of Münster, Institute of Inorganic and Analytical Chemistry and NRW Graduate School of Chemistry, Corrensstr. 30, 48149 Münster, Germany*
[b] *University of Leipzig, Institute of Pharmacology, Pharmacy and Toxicology, Faculty of Veterinary Medicine, An den Tierkliniken 15, 04103 Leipzig, Germany*
[c] *University of Leipzig, Rudolf-Boehm-Institute of Pharmacology and Toxicology, Clinical Pharmacology, Faculty of Medicine, Haertelstr. 16-18, 04107 Leipzig, Germany*

HIGHLIGHTS

- Biotransformation pathways of the two *Amaryllidaceae* alkaloids galantamine and lycorine were investigated.
- Three different methods were used and compared including an *in vivo*, *in vitro* and electrochemical (EC) approach.
- It was possible to predict most of the metabolites observed during conventional plasma and microsomal studies with EC.
- Dealkylation, dehydrogenation and oxygenation were found to be the main metabolic routes of both alkaloids.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 September 2012
Received in revised form 17 October 2012
Accepted 22 October 2012
Available online 1 November 2012

Keywords:
Amaryllidaceae alkaloids
Galantamine
Metabolism
Electrochemistry
Liquid chromatography
Mass spectrometry

ABSTRACT

Alkaloids from the plant family of *Amaryllidaceae*, such as galantamine (GAL) and lycorine (LYC), are known to exhibit numerous promising biological and pharmacological activities like antibacterial, antiviral or anti-inflammatory effects. Nonetheless, studies on the biotransformation pathway are rare for this substance class, unless approval for use as medication exists. While GAL has become a prescription drug used to alleviate and delay the symptoms of Alzheimer's disease, LYC exhibits potential antitumor properties. However, it has also been linked to toxic effects resulting in nausea and emesis. Whereas there are few publications available describing the metabolic pathway of GAL in animals and humans, the metabolism of LYC is unknown. Therefore, this study is concerned with the investigation of the oxidative metabolism of GAL and LYC, which was achieved by means of three different approaches: electrochemical (EC) simulation coupled on-line to liquid chromatography (LC) with electrospray mass spectrometric (ESI-MS) detection was applied in addition to *in vivo* experiments in beagle dog analyzing plasma (BP) and *in vitro* incubations with rat liver microsomes (RLM). This way, it should be investigated if electrochemistry can be used to predict the oxidative metabolism of alkaloids. For GAL, the EC model was capable of predicting most metabolites observed during microsomal and plasma studies, including *N*-demethylated, dehydrogenated and oxygenated products or a combination of these. LYC was found to be metabolized

* Corresponding author at: Institute of Inorganic and Analytical Chemistry, University of Münster, Corrensstr. 30, 48149 Münster, Germany. Tel.: +49 251 8333141; fax: +49 251 8336013.
E-mail addresses: sandra.jahn@uni-muenster.de (S. Jahn), bettina.seiwert@ufz.de (B. Seiwert), sascha.kretzing@gmx.de (S. Kretzing), gabraham@vetmed.uni-leipzig.de (G. Abraham), ralf.regenthal@medizin.uni-leipzig.de (R. Regenthal), uk@uni-muenster.de (U. Karst).

0003-2670/$ – see front matter
http://dx.doi.org/10.1016/j.aca.2012.10.042

far less than GAL in the animal-based approaches, but several EC oxidation products were generated. Some principal metabolic routes could successfully be correlated for this alkaloid as well, comprising dehydrogenation, dehydration to ungeremine and oxygenation reactions.

1. Introduction

Galantamine (or galanthamine, GAL, Fig. 1a) and lycorine (LYC, Fig. 1b) are two tertiary, chiral alkaloids that can be extracted from different *Amaryllidaceae* species including bulbs and flowers of daffodil (*Narcissus*), snowdrop (*Galanthus*) or spider lily (*Lycoris*). *Amaryllidaceae* alkaloids exhibit various advantageous biological and pharmacological activities rendering them interesting for the use as potential therapeutics [1]. Especially GAL is already employed in treating the symptoms of mild to moderate Alzheimer's disease and other memory impairments like dementia [2,3]. Additionally, it is utilized for neuromuscular diseases and blockades [4,5] or to antagonize drug-induced respiratory depression [6]. GAL mainly acts by reversible inhibition of acetylcholinesterase (AChE) and has been approved as a prescription drug by the FDA in 2001 [7,8]. In comparison, LYC displays the most frequent *Amaryllidaceae* alkaloid and has been ascribed a number of biological effects such as antiviral [9], antifungal, anti-inflammatory [10] and, in particular, anticancer properties [1,11,12]. Furthermore, it has been shown to weakly inhibit AChE [13], protein synthesis [14] and ascorbic acid biosynthesis [15]. On the other hand, a recent dose-dependent *in vivo* study with beagle dogs revealed that LYC is directly associated with the cause of nausea and emesis in animals upon poisoning owing to ingestion of plant material from the *Amaryllidaceae* kind [16]. In a subsequent examination, the same authors were able to reveal the causative involved targets of LYC-induced emesis [17]. Therefore, it is rather astonishing that the metabolic pathway of this alkaloid in humans and animals is largely unknown until now, particularly, when considering the effort which has been directed toward the evaluation of LYC as a potential antitumor agent [18,19]. Merely the biochemical significance and metabolism of LYC in fruits of *Crinum asiaticum* and in flower-stem fluid of *Crinum latifolium* have been reported by Ghosal et al. [20,21]. The biotransformation of GAL has already been investigated in rats, dogs and humans, but only a limited number of metabolic studies are available so far [8,22,23]. Principal phase I metabolic pathways for GAL comprise *O*- and *N*-demethylation, *N*-oxidation as well as epimerization. Furthermore, phase II metabolism reactions like glucuronidation and sulfate conjugation have been described.

Traditionally, the biotransformation of pharmaceutically promising substances is evaluated on the basis of *in vivo* and *in vitro* methods with laboratory animals and hepatic cells, cell extracts or liver cell microsomes [24]. The liver contains the largest quantity of cytochrome P450 (CYP) enzymes, which are mainly responsible for metabolizing xenobiotics in an organism [25,26]. These approaches are well-established and results often show good agreement with the processes observed in the human body. However, they are quite time-consuming, laborious and costly. Moreover, if animal-based models are used, direct transferability cannot be claimed for *in vivo* and *in vitro* experiments, because of variations in the distribution of the P450 subfamilies. In an attempt to initially diminish the difficulties regarding complexity and time exposure, electrochemistry (EC) coupled to liquid chromatography (LC) and electrospray mass spectrometry (ESI-MS) has been introduced as a complementary, purely instrumental approach to mimic oxidative metabolism reactions [27,28]. Within the last three decades, research in this area has rapidly advanced, currently enabling a significant amount of CYP-mediated oxidation reactions to be simulated in a fast and simple manner [29,30]. The employment of novel electrode materials such as boron-doped diamond (BDD) [31], different cell types or specifically optimized oxidation conditions [32] contributed to this development. Although the EC-based method can certainly not be a full surrogate for *in vivo* and *in vitro* investigations, it is still an extremely helpful and rapid tool in providing preliminary ideas of what oxidative metabolites can be formed. With some exceptions [33–35], a majority of the work in this scientific field was dedicated to the analysis of classical pharmaceuticals such as diclofenac [36,37], tetrazepam [38] or verapamil [39], for instance. However, nothing has been reported so far on using the on-line EC/LC/ESI-MS method for mimicking the oxidative biotransformation pathway of alkaloids or related compounds which will be addressed in the present investigation.

In view of the limited data on the metabolic fate of pharmacologically promising alkaloids, this work is concerned with metabolic studies on the two *Amaryllidaceous* alkaloids GAL and LYC. These were achieved by means of electrochemical simulation along with conventional *in vivo* and *in vitro* approaches. Regarding the *in vivo* experiments, plasma from beagle dogs (BP) treated with the alkaloid of interest was analyzed *via* LC/ESI-MS, whereas rat liver microsomal incubations (RLM) served as *in vitro* metabolizing system. The results were compared for each alkaloid with respect to the different method applied and (if available) with data from the literature. Thereby, it should be investigated if phase I metabolites found in animal-based models can be predicted by the EC approach. Structural conclusions were drawn by reference to accurate mass data and MS/MS product ion scans. The set-up of the on-line EC/LC/ESI-MS system applied in this study has previously been described including a schematic plot [39].

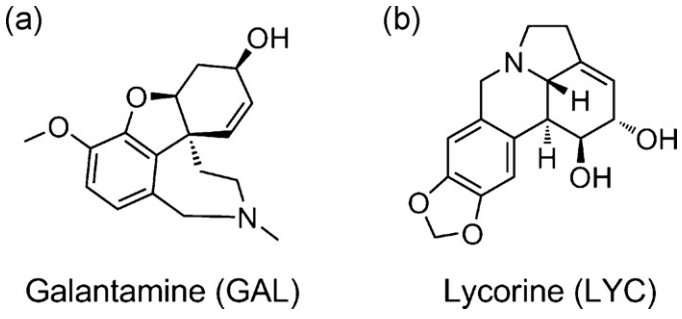


Fig. 1. Structural formulae of the investigated alkaloids (a) galantamine (GAL) and (b) lycorine (LYC).

2. Experimental

2.1. Chemicals

Lycorine hydrochloride (LYC), L-glutathione (GSH), ammonium acetate (NH_4Ac), ammonia solution (25%) and magnesium chloride hexahydrate were delivered by Sigma Aldrich Chemie GmbH (Steinheim, Germany). Acetic acid (HAc), formic acid (FA), sodium dihydrogen phosphate dihydrate, and disodium hydrogenphosphate were purchased from Fluka Chemie GmbH (Buchs, Switzerland). L-Cysteine (Cys) was acquired from Acros Organics (Geel, Belgium) and galantamine hydrobromide (GAL) was