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MECHANISTIC STUDY OF COLCHICINE's ELECTROCHEMICAL OXIDATION

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ABSTRACT

Colchicine, as one of the most ancient drugs of human kind, is still in the focal point of the current research due to its multimodal mechanism of action. The elucidation of colchicine's still unknown redox properties may play an important role in deciphering its beneficial and harmful implications over the human body. Therefore, a systematic mechanistic study of colchicine's oxidation has been undertaken by electrochemistry coupled to mass spectrometry using two different types of electrolytic cells, in order to clarify the existing inconsistencies with respect to this topic. At around 1 V vs. Pd/H_2, initiated by a one-electron transfer, the oxidation of colchicine sets off leading to a cation radical, whose further oxidation may evolve on several different pathways. The main product of the anodic electrochemical reaction, regardless of the carrier solution's pH is represented by a 7-hydroxy derivative of colchicine. At more anodic potentials (above 1.4 V vs. Pd/H_2) compounds arising from epoxidation and/or multiple hydroxylation occur. No di- or tridemethylated quinone structures, as previously suggested in the literature for the electrolytic oxidation of colchicine, has been detected in the mass spectra.

1. Introduction

Colchicine, N-[(7S)-1,2,3,10-tetramethoxy-9-oxo-5,6,7,9-tetrahydrobenzo(a) heptalen-7-yl]acetamide (**Col**), the main proto-alkaloid of the poisonous plant meadow saffron (Colchicum autumnale L.), is a specific anti-inflammatory agent used for centuries in the treatment of acute gouty arthritis. In the last decade, it has also proven its efficiency in the treatment of Mediterranean fever, Behçet's syndrome, scleroderma, amyloidosis, liver cirrhosis, but also other comorbid conditions associated with gout, such as osteoarthritis and various cardiovascular diseases [1,2]. **Col** demonstrates a multimodal mechanism of action and continues to be in the focal point of the very recent biomedical, clinical and toxicological research [2–7]. Although **Col** is not used clinically to treat cancer due to its toxicity, it does exert anti-proliferative effects through the inhibition of microtubule formation leading to mitotic arrest and cell death by apoptosis. However, it has also been shown to produce anti-vascular effects [8] leading to a greater reduction of blood flow in tumors than in normal tissues and the ability to overcome P-glycoprotein efflux pump mediated multidrug resistance [9], which renders **Col** a model molecule for the development of a series of novel anticancer drugs with a better toxicological profile [1,10–13], many of them currently undergoing clinical studies. Nevertheless, as a downside, it has also been reported that the central administration of cytoskeletal poisons, such as **Col**, causes oxidative stress in animals leading to cognitive impairment [14], and its therapeutic use has been linked to sporadic Alzheimer's disease in humans [15].

Therefore, it is obvious that the determination of **Col** content in various biological samples is particularly important, for which various high performance separation techniques became available [16–19]. As a more cost- and time-effective alternative, several highly sensitive electroanalytical methods have also been described [20–28].

Since the first assumptions made by Woodson in 1970 and a few years later by Bishop [29,30] related to the redox mechanism of **Col**, not much has been done with respect of the experimental confirmation of the assumptions. Nevertheless, recently a systematic study of **Col**'s reduction mechanism has been reported by our group, clarifying the existing inconsistencies and correlating the findings with some of its reported toxicological effects [31]. The elucidation of **Col**'s redox properties could play a crucial role in the confirmation and prediction of some of its relevant pharmacokinetic, pharmacodynamic and toxicological properties and

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ultimately a parallel may be drawn with its reported in vivo oxidative biotransformation [32].

The redox behavior of many natural or synthetic bioactive compounds has been studied by electrochemistry (EC) hyphenated with other various analytical techniques, but by far the most advantageous paring is with mass spectrometry (MS) [33,34]. Since its first report in 1971 [35], the on-line EC/MS coupling was predominantly exploited for the identification of the emerging stable and/or transient (radical-cation or radical-anion) reaction products [36–39]. Nowadays, EC/MS grew into a powerful technology of wide applicability, such as: signal enhancement in MS, proteomics, study of DNA damage, testing of cosmetics on skin sensitizing effects, predicting the degradation and persistence of pollutants, and last, but not least, mimicking drug and xenobiotic metabolism [40,41].

Using EC as a reaction technique before the MS allows the simulation of natures' redox reactions in a controlled manner. Numerous biochemical reactions (enzymatic, microbial, etc.) can be simulated without biological interferences, making EC/MS a real biomimetic tool with substantial cost and time savings compared to in-vivo or in-vitro techniques. Significant aspects reported up to now about the biotransformation of **Col** is that is undergoing an intestinal and hepatic oxidative metabolism by cytochrome P450 (CYP) 3A4 isoform [2] generating demethylated metabolites (2-demethylcolchicine, 3-demethylcolchicine and 10-demethylcolchicine [32]. **Col** is also the substrate for P-glycoprotein 1 efflux transporter, regulating the protoalkaloid's tissue distribution, responsible of its enterohepatic re-circulation and renal excretion [3].

Therefore, the aim of the study was to undertake for the first time a systematic mechanistic study of **Col**'s electrochemical oxidation, to fill out the existing knowledge gaps and clarify the inconsistencies and, finally to correlate the findings with its reported oxidative biotransformation.

2. Experimental

2.1. Chemicals and standards

Analytical grade **Col** for biochemistry was purchased from Merck, Germany. Aqueous stock solutions were freshly prepared each day and they were stored in the dark. Working standards of **Col** were prepared adding appropriate amounts of stock solution directly into the working electrolyte. Aqueous and non-aqueous solutions of supporting electrolytes were prepared by dissolving the appropriate amounts in ultrapure water or the given solvent.

Phosphoric acid (98%, w/w), N,N-dimethylformamide, Suprapur grade hydrochloric acid (30%, w/w) and silver nitrate were purchased from Merck, Germany. Formic acid (96%, w/w) and tetrabutylammonium hexafluorophosphate (TBAHFP) were obtained from Fluka, Switzerland. Reagent grade sodium hydroxide, potassium nitrate, acetic acid (>99%, v/v), ammonium acetate, boric acid (>99.5%) and ammonium hydroxide solution (28-30%,v/v) were purchased from Sigma-Aldrich, Germany, whereas Optigrade acetonitrile (ACN) was purchased from Promochem (Germany).

2.2. Apparatus

All electrochemical assays were performed with a modular PGSTAT30 potentiostat (Metrohm Autolab BV, The Netherlands) using its dedicated GPES 4.9 software for data acquisition and subsequent processing. Cyclic voltammetry, fast scan voltammetry and chronoamperometry were performed in a standard 15 mL electrochemical cell with a classical three-electrode setup. Glassy carbon electrode (d = 3 mm) as working electrode, Ag/AgCl (3 M KCl) as reference and a platinum wire as auxiliary electrode were used. For non-aqueous media Ag/Ag^+ reference electrode (0.01 M $AgNO_3$, 0.1 M TBAHFP in the given solvent) was employed. The pH dependence of the electrochemical process was assessed in 0.05 M Britton-Robinson buffer adjusted with 0.1 M NaOH.

2.3. EC-MS analysis

Col dissolved in the corresponding aqueous or non-aqueous media has been infused with the aid of a syringe pump (SP2, Antec, The Netherlands) by 1/16" o.d. PEEK tubing through the electrochemical cell into an ESI-MS system (Agilent 6300 LC/MSD Ion Trap SL, Agilent Technologies) at a flow rate of 15-33.33 μL min^{-1} while the ionization (both +ESI and –ESI) and mass spectrometric detection parameters were kept constant, working in a full scan mode on the range of m/z = 100-900. Two types of electrochemical cells, connected with the ESI-MS system in the decoupled mode, were tested: a two-compartment cell made in-house, with a dialysis membrane separating the working electrode's compartment (glassy carbon + carbon felt) from the reference and auxiliary electrode's compartment, as reported by Arakawa [38] and a one-compartment cell (ReactorCell, Antec, The Netherlands), with a diamond based working electrode (Magic Diamond™), carbon loaded PTFE inlet block as auxiliary and Pd/H_2

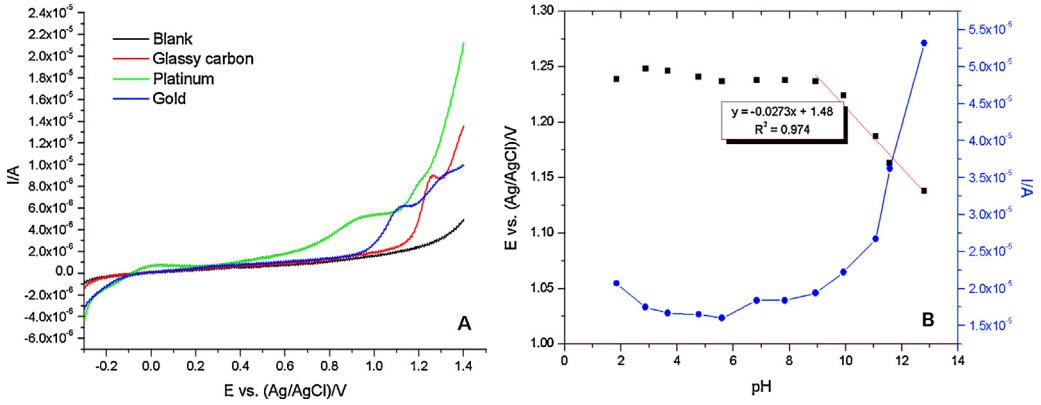

Fig. 1. A Oxidation of 0.3 mM colchicine in 0.1 M KNO_3 (pH = 6.25) using glassy carbon (red), platinum (green) and gold (blue) electrode. Blank assay using glassy carbon electrode (black). Glassy carbon (E_{ox1} = 1.26 V, E_{ox2} = 1.38 V), platinum (E_{ox1} = 0.91 V, E_{ox2} = 1.19 V), gold (E_{ox1} = 1.12 V, E_{ox2} = 1.32 V). Anodic linear sweep voltammetry 50 mV s^{-1}. B The pH dependence of peak potential and current intensity in the oxidation of colchicine on glassy carbon electrode. Anodic linear sweep voltammetry 100 mV s^{-1}, 0.3 mM colchicine in 0.05 M Britton-Robinson buffer adjusted with 0.1 M NaOH; Ag/AgCl (3 M KCl) reference electrode; Pt wire auxiliary electrode. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)