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Genotoxicity and cytotoxicity of oxindole alkaloids from *Uncaria tomentosa* (cat's claw): Chemotype relevance

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Mitraphylline (PubChem CID: 94160)
Isomitraphylline (PubChem CID: 11726520)
Speciphylline (PubChem CID: 168985)
Uncarine F (PubChem CID: 12304288)
Pteropodine (PubChem CID: 10429112)
Isopteropodine (PubChem CID: 9885603)
Rhynchophylline (PubChem CID: 5281408)
Isorhynchophylline (PubChem CID: 3037048)

ABSTRACT

Ethnopharmacological relevance: *Uncaria tomentosa* (Willdenow ex Roemer & Schultes) DC. (Rubiaceae) or cat's claw is a climber vine from the South American rainforest used in folk medicine for cancer treatment. Its antitumor activity has been mostly ascribed to pentacyclic oxindole alkaloids (POA) from stem bark and leaves while the activity of tetracyclic oxindole alkaloids (TOA) remains unknown. In recent times, the occurrence of three chemotypes based on its oxindole alkaloid profile was noticed in *U. tomentosa*, namely, chemotype **I** (POA *cis* D/E ring junction); chemotype **II** (POA *trans* D/E ring junction) or chemotype **III** (TOA). Consequently, the relationship between the chemotype and cytotoxic and genotoxic activities deserves attention.

Aim of the study: To evaluate the influence of cat's claw chemotypes on genotoxicity and cytotoxicity against non malignant and malignant human cell line models.

Material and methods: Four authentic stem bark cat's claw samples (S_I-S_{IV}) and two leaf samples (L_{II} and L_{III}) were analyzed by HPLC-PDA, properly extracted and fractioned by ion-exchange to obtain oxindole alkaloid purified fractions (OAPFs). The freeze-dried fractions were assayed for genotoxicity and cytotoxicity against human leukocytes (non malignant cell line) by the micronuclei frequency method and the alkaline comet DNA assay, and the trypan blue method, respectively. Moreover, the cytotoxicity of each OAPF was evaluated against a human bladder cancer cell line (T24) and human glioblastoma cell line (U-251-MG) by MTT method (malignant cell lines). Additionally, the isomerization of oxindole alkaloids throughout the course of cell incubation was monitored by HPLC-PDA.

Results: Based on HPLC-PDA analyses, sample S_I was characterized as chemotype **I**, while samples S_{II} and L_{II} were characterized as chemotype **II**, and samples S_{III}, S_{IV} and L_{III} as chemotype **III**. The chemotypes showed comparable cytotoxic activity toward malignant cell lines (T24 and U-251-MG) unlike human leukocytes (non malignant cell line), where this activity was clearly distinct. Chemotype **II** (POA *trans* D/E ring junction) showed a higher selectivity index (SI) against malignant cells (SI = 1.11–3.04) than chemotype **I** (SI = 0.10 – 0.19) and **III** (SI = 0.21–0.57). No important genotoxic potential was found by micronuclei frequency and alkaline comet DNA assays. Despite the isomerization of oxindole alkaloids during the cell incubation, the chemotype of the cat's claw samples remained unchanged.

Conclusion: Cat's claw chemotypes showed different selectivity against human malignant cells, so that the correct identification of each chemotype seems to be important to better understand its antitumor potential.

1. Introduction

Uncaria tomentosa (Willdenow ex Roemer & Schultes) DC. (Rubiaceae), popularly known as cat's claw or "Uña de Gato", is a climber vine from the South American rainforest traditionally employed as immunostimulant, anti-inflammatory and also for cancer treatment (Heitzman et al., 2005; Keplinger et al., 1999;

Abbreviations: POA, pentacyclic oxindole alkaloids; TOA, tetracyclic oxindole alkaloids; OAPFs, oxindole alkaloid purified fractions; T24, human bladder cancer cell line; U-251-MG, human glioblastoma cell line; SI, selectivity index; SPEC, speciophylline; UNCF, uncarine F; PTER, pteropodine; ISPTER, isopteropodine; MIT, mitraphylline; ISMITR, isomitraphylline; RHY, rhynchophylline; ISRHY, isorhynchophylline; S, stem bark samples; L, leaf samples; CE, crude extract

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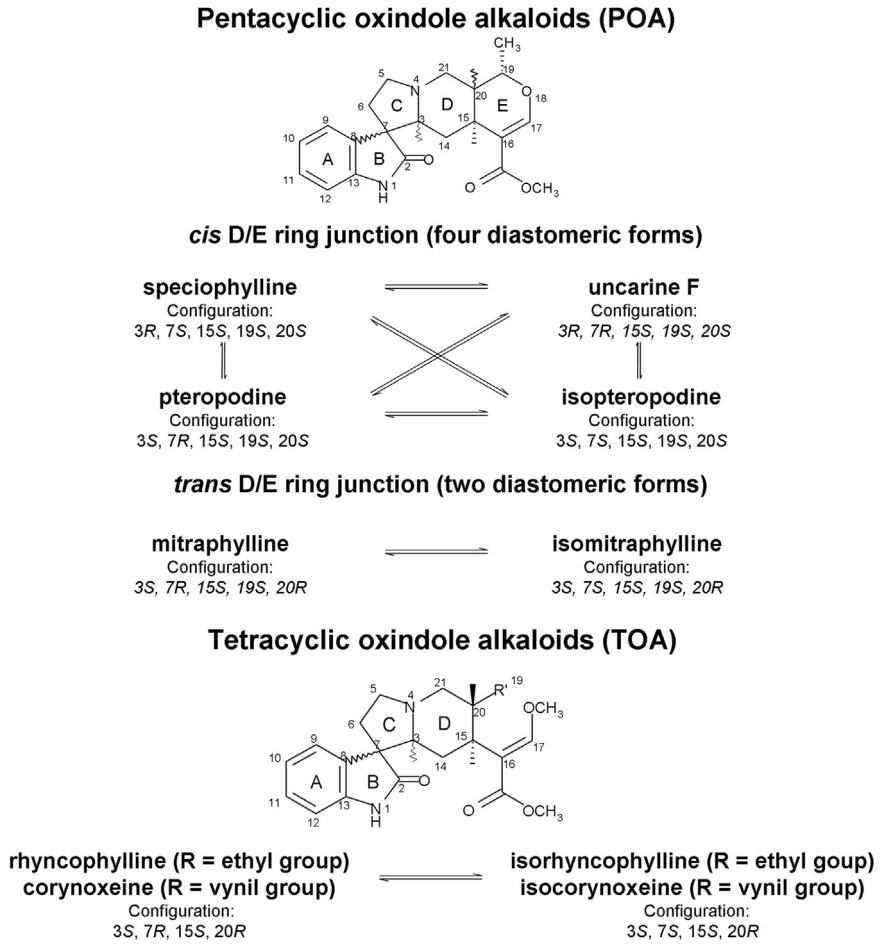

Fig. 1. Main oxindole alkaloids reported in cat's claw and their isomerization process.

Zhang et al., 2015). Antitumor activity has been mostly ascribed to its stem bark oxindole alkaloids (Heitzman et al., 2005; Kaiser et al., 2013a; Pilarski et al., 2010), which can occur as pentacyclic (POA) or tetracyclic (TOA) derivatives (Fig. 1). Both POA and TOA are susceptible to isomerization. The isomerization rate depending on pH, temperature and medium polarity (Laus et al., 1996). For POA with *trans* D/E ring junction, isomerization leads to one pair of interconvertible diastereomeric forms (mitraphylline and isomitraphylline), while for POA with *cis* D/E ring junction undergoes isomerization to four interconvertible diastereomeric forms (speciophylline, uncarine F, pteropodine and isopteropodine) (Laus et al., 1996). Concerning TOA, two pairs of interconvertible diastereomeric forms are found, which differ in the group attached at C-20 (ethyl group – rhyncophylline and isorhyncophylline; vinyl group – corynoxeine and isocorynoxeine) (Laus et al., 1998).

The occurrence of two chemotypes in cat's claw based on its oxindole alkaloid profile (chemotype POA or TOA) was first reported by Laus et al. (1997). Recently, three specific chemotypes were found from the study of the chemical variability of a wild population of cat's claw from the Peruvian Amazon (Peñaloza et al., 2015). These chemotypes were named as chemotype **I**, composed mainly by POA with *cis* D/E ring junction; chemotype **II**, composed mainly by POA with *trans* D/E ring junction; and chemotype **III** composed mainly by TOA.

The occurrence of an antagonistic effect between TOA and POA has been reported. POA was able to stimulate the endothelial cells *in vitro* to produce a lymphocyte-proliferation-regulating factor, while the increased TOA concentration in the medium inhibited the factor production (Keplinger et al., 1999; Wurm et al., 1998). Probably based on these findings, the U. S. Pharmacopeia limited the TOA content to 0.05% (w/w) of the dried raw material, and up to 25% (w/w) in relation to the POA content in the cat's claw derivatives such as powdered dried extract, capsules and tables (USP, 2016). On the other hand, against human peripheral blood mononuclear cells (PBMC) stimulated with phytohaemagglutinin and concanavalin A, both POA and TOA showed similar suppression activity in immunobiochemical pathways induced by interferon-γ (Winkler et al., 2004). Thus, the antagonistic effect of TOA in relation to POA needs to be clarified.

Cat's claw preparations are considered effective and safe as a phytomedicine for human use, without major restrictions regarding their acute toxicity, genotoxicity or cytotoxicity (Keplinger et al., 1999; Romero-Jiménez et al., 2005; Valerio Jr and Gonzales, 2005). Despite the antitumor activity found for cat's claw quinovic acid glycosides (Dietrich et al., 2014), it has been mostly reported for the cat's claw oxindole alkaloids (Heitzman et al., 2005; Kaiser et al., 2013a; Pilarski et al., 2010; Zhang et al., 2015). However the effect of the different oxindole alkaloid profiles found in cat's claw on antitumor activity remains unknown. Thus, the present study aims to evaluate the influence of the cat's claw chemotypes on the genotoxicity and cytotoxicity against human non malignant cell line (human leukocytes) and cytotoxicity against human malignant cell lines (T24 human bladder cancer cell line and U-251-MG human glioblastoma cell line).