Fitoterapia 127 (2018) 101–108

Contents lists available at ScienceDirect

Fitoterapia

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

Inhibition of aldose reductase activity by *Cannabis sativa* chemotypes extracts with high content of cannabidiol or cannabigerol

Antonella Smeriglio[a,b,*], Salvatore V. Giofrè[a], Enza M. Galati[a], Maria T. Monforte[a], Nicola Cicero[c], Valeria D'Angelo[a], Gianpaolo Grassi[d], Clara Circosta[a]

[a] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98168 Messina, Italy
[b] Foundation Prof. Antonio Imbesi, University of Messina, Messina, Italy
[c] Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, 98166 Messina, Italy
[d] CRA-Research Institute for Industrial Crops (ISCI), 45100 Rovigo, Italy

ARTICLE INFO

Keywords:
Cannabis sativa L. chemotypes
Standardized extracts
Non-psychotropic phytocannabinoids
Aldose reductase inhibitory activity
Molecular docking study

ABSTRACT

Aldose reductase (ALR2) is a key enzyme involved in diabetic complications and the search for new aldose reductase inhibitors (ARIs) is currently very important. The synthetic ARIs are often associated with deleterious side effects and medicinal and edible plants, containing compounds with aldose reductase inhibitory activity, could be useful for prevention and therapy of diabetic complications. Non-psychotropic phytocannabinoids exert multiple pharmacological effects with therapeutic potential in many diseases such as inflammation, cancer, diabetes. Here, we have investigated the inhibitory effects of extracts and their fractions from two *Cannabis sativa* L. chemotypes with high content of cannabidiol (CBD)/cannabidiolic acid (CBDA) and cannabigerol (CBG)/cannabigerolic acid (CBGA), respectively, on human recombinant and pig kidney aldose reductase activity *in vitro*.

A molecular docking study was performed to evaluate the interaction of these cannabinoids with the active site of ALR2 compared to known ARIs.

The extracts showed significant dose-dependent aldose reductase inhibitory activity (> 70%) and higher than fractions. The inhibitory activity of the fractions was greater for acidic cannabinoid-rich fractions. Comparative molecular docking results have shown a higher stability of the ALR2-cannabinoid acids complex than the other inhibitors. The extracts of *Cannabis* with high content of non-psychotropic cannabinoids CBD/CBDA or CBG/CBGA significantly inhibit aldose reductase activity. These results may have some relevance for the possible use of *C. sativa* chemotypes based preparations as aldose reductase inhibitors.

1. Introduction

Aldose reductase (ALR2) is the first enzyme of the polyol pathway that catalyzes the reduction of glucose to sorbitol utilizing NADPH as a cofactor. The intracellular accumulation of sorbitol, due to increased aldose reductase activity at high blood glucose levels, such as those occurring in diabetes, has been implicated in the development of various secondary complications of diabetes such as neuropathy, nephropathy, retinopathy, and cataract, which practically are not controlled by insulin [1]. The aldose reductase inhibitors (ARIs) can prevent the reduction of glucose to sorbitol and reduce complications of diabetes [2]. The synthetic ARIs are often associated with deleterious side effects and poor penetration of target tissues such as nerve and retina [3]. Numerous natural compounds such as flavonoids, coumarins, terpenes and related aromatic compounds have been reported in the literature to have *in vitro* aldose reductase inhibitory activity [4,5]. Plants are a very important source of substances of high chemical diversity, many of which exert interesting pharmacological activities. *Cannabis sativa* L. (Cannabaceae) has been an important source of food, fiber, dietary oil and medicine for thousands of years in Europe, Asia and Africa [6–9]. Several epidemiological studies have independently associated the cannabis use with a lower prevalence of diabetes mellitus (DM) in past

Abbreviations: ALR2, aldose reductase; ARIs, aldose reductase inhibitors; Δ^9-THC, Δ^9-tetrahydrocannabinol; CBD, cannabidiol; CBG, cannabigerol; THCA, Δ^9-tetrahydrocannabinolic acid; CBDA, cannabidiolic acid; CBGA, cannabigerolic acid; RSD, relative standard deviation; NADPH, nicotinamide adenine dinucleotide phosphate; DMSO, dimethyl sulfoxide; ARHR, aldose reductase human recombinant; ARPK, aldose reductase pig kidney; $NADP^+$, nicotinamide adenine dinucleotide phosphate; RMSD, root mean square deviation; NPA, Nose-Poincare-Anderson

* Corresponding author at: Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, 98168 Messina, Italy.
E-mail address: asmeriglio@unime.it (A. Smeriglio).

https://doi.org/10.1016/j.fitote.2018.02.002
Received 4 December 2017; Received in revised form 22 January 2018; Accepted 3 February 2018
Available online 07 February 2018
0367-326X/

and present cannabis consumers compared to non-cannabis users [10]. Cannabis plant is characterized by the presence of terpenophenolic compounds, known as cannabinoids. *C. sativa* chemotypes distinction, is based mainly on the content of Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol, cannabigerol, and their acidic forms tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA) and cannabigerolic acid (CBGA). The well-known psychotropic effects of Δ^9-THC, have greatly limited therapeutic use of *Cannabis*. However, the presence in *C. sativa* of non-psychotropic cannabinoids could be promising for its possible use in therapy, free of the side psychotropic effects. These compounds include CBD, CBG, as well as cannabinoid acids such as CBDA and CBGA. The most promising of the non-psychotropic cannabinoids is CBD, which exerts several positive pharmacological effects that make it a highly attractive therapeutic entity in multiple disease models, [11–13] including diabetes [14,15] and diabetic complications [16–19].

In this study, the inhibitory effects on aldose reductase activity by hexane extracts and their fractions from two different chemotypes of *C. sativa* fiber type CBD- or CBG-rich, with a content of Δ^9-THC < 0.2%, were evaluated *in vitro*.

Phytochemical analyses of extracts and fractions were carried out by HPLC-DAD, ^{1}H NMR and GC–MS methods. Molecular docking studies were performed to evaluate the interaction of non-psychotropic cannabinoids, CBD/CBDA and CBG/CBGA, with the active site of ALR2 comparing them with other known inhibitors.

2. Materials and methods

2.1. Chemicals

Acetonitrile and formic acid HPLC-grade were purchased from Merck (Darmstadt, Germany). CBD, CBG, CBDA, CBGA and quercetin analytical standards (≥95%) as well as acetic acid, hexane, NADPH, DL-glyceraldehyde, phosphate buffer saline, DMSO and human recombinant aldose reductase (ARHR) were purchased from Sigma-Aldrich (Milan, Italy).

2.2. Plant material and sample preparation

Flowering tops dried of two different chemotypes of *Cannabis sativa* L. of the fiber type named CBD-type (batch number 12) and CBG-type (batch number 21), respectively, were provided by the CRA-Research Centre for Industrial Crops (ISCI) of Rovigo (Italy).

Extracts preparation was carried out according to Hazekamp et al. [20] with some modifications. Briefly, 50 g of CBD- and CBG-type cannabis flowering tops dried were extracted three times with 500 ml of acetic acid/hexane 0.1% v/v, sonicating for 5 min (USC300TH, VWR) and then proceeding the extraction procedure under constant agitation for 3 h at room temperature and in the dark. The hexane extracts were combined, filtered and dried by a rotary evaporator (Buchi R-205, Cornaredo, Italy) obtaining a yield of 11.6 and 9.6%, respectively. The dried extracts were stored at −20 °C until analyses.

2.3. Phytochemical profile of cannabis extracts by GC–MS

Cannabis hexane extracts were injected into an Agilent 7890 B GCMS system, equipped with an autosampler G4513A Agilent and a split/splitless injector. The column used was a SLB-5MS (Supelco, Milan, Italy), 30 m × 0.25 mm i.d., 0.25 μm df, coated with 5% diphenyl-95% polydimethylsiloxane, operating with the following oven temperature programme: 50 °C, held for 1 min, rising at 3 °C/min to 250 °C, held for 5 min, rising at 10 °C/min to 270 °C, held for 3 min; injection temperature and volume, 250 °C and 1.0 μl, respectively; injection mode, split; split ratio, 50:1; carrier gas, helium at 30 cm/s linear velocity and inlet pressure 99.8 KPa. Mass spectrometer parameters were as follows: interface and source temperatures, 250 °C and 200 °C, respectively; ionization mode, EI with an energy 70 (eV) applied; acquisition mass range, 40–400 *m/z* with a scan speed of 1666 amu/s and a scan interval of 0.3 s. Data handling was supported by the software MassHunter Qualitative Analysis B. 07.00 (Agilent). The compounds were identified based on values reported in the literature, the computer matching of their mass spectral data with those of the MS libraries (NIST 14 search 2.2 and Wiley), the study and comparison of their MS fragmentation patterns with those reported in literature, and, whenever possible, the co-injection with authentic standards (CBD, CBG).

2.4. Determination of main acidic and neutral phytocannabinoids by HPLC-DAD analysis

Qualitative and quantitative analysis of acidic and neutral cannabinoids was performed according to Gul et al. [21] with some modifications, using an Agilent high performance liquid chromatography system (1100 series) equipped with a UV–vis photodiode-array detector (DAD) (G1315) coupled with a control system (G1323), a binary pump (G1312) and an auto-injector (G1313). The chromatographic separation was carried out on Eclipse reversed phase column (150 × 4.6 mm, 5 m; Agilent), using as eluent the solvent A (water acidified with 0.1% formic acid) and solvent B (acetonitrile) according to the following elution program: 0–6 min, 70% B; 6–12 min, 77% B; 12–22 min, 77% B and equilibrated for 2 min for a total run time of 25 min. The flow rate was set at 1.5 ml/min, the column maintained at 28 °C and the injection volume set to 10 μl. The UV-VIS spectra of phytocannabinoids of interest were recorded in the range 190–400 nm and the chromatograms acquired at 220 nm. The identity of the compounds of interest was confirmed comparing their retention times and UV-VIS spectra with those of the reference standard commercially available. Quantification was carried out by external standard calibration curves.

2.5. Separation of cannabinoid-rich fractions by MPLC

For the separation of cannabinoid-rich fractions was used a MPLC device equipped with a Biotage column Snap Cartridge KP-Sil 50 g, using a mobile phase chloroform/methanol 99:1 (v/v). The flow rate was set at 5 ml/min. The sample (1 g of the hexane extract CBD- or CBG-type, respectively) was dissolved in chloroform until a final volume of 4 ml for injection. Eight fractions, each one of 10 ml, were collected.

Each fraction was analyzed by TLC and selected fractions were further analyzed by HPLC and confirmed by ^{1}HNMR. Fractions containing a high proportion (> 90%) of a single cannabinoid (CBD or CBDA, CBG or CBGA) were combined, evaporated to dryness and kept at −20 °C for analyses.

2.6. NMR analysis

The non-psychotropic phytocacannabinoid-rich fractions were analyzed by nuclear magnetic resonance to confirm cannabinoids identity and to detect any remaining impurities. The samples were dissolved in deuterated chloroform ($CDCl_3$) at 10 mg/ml concentration. Spectra ^{1}H NMR were recorded with a Varian 500 MHz (Agilent Technologies, Palo Alto, CA, USA) using the chemical shift of CDCl3 at 7:26 ppm as reference. The number of scans were 128 or 64. Spectra data were consistent with a previous reports [22,23]. Sample mass was determined by TSQ Quantum XLS Triple Quadrupole GC–MS/MS (Thermo Scientific, Waltham, MA, USA).

2.7. Aldose reductase inhibition assays

2.7.1. Aldose reductase pig kidney (ARPK) preparation

Aldose reductase was prepared from fresh pig kidney [24] obtained commercially. The tissue was dissected from the connective tissue,