Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 14 (2007) 333–337
Phytomedicine
www.elsevier.de/phymed

Anticoagulant effects of a *Cannabis* extract in an obese rat model

C. Coetzee, R.-A. Levendal, M. van de Venter, C.L. Frost*

Department of Biochemistry and Microbiology, Nelson Mandela Metropolitan University, PO Box 77000, Port Elizabeth 6031, South Africa

Abstract

Blood coagulation studies were conducted to determine the possible anti-/prothrombotic effect of an organic cannabis extract and the three major cannabinoids, THC, CBD and CBN. The in vitro effect of the cannabis extract on thrombin activity produced an IC_{50} value of 9.89 mg/ml, compared to THC at 1.79 mg/ml. It was also found that the extract, THC and CBN showed considerable inhibition of thrombin-induced clot formation in vitro with IC_{50} values of 600, 87 and 83 μg/ml for the extract, THC and CBN respectively. In an in vivo model used to determine clotting times of lean and obese rats treated with a cannabis extract, 50% clotting times were found to be 1.5 and 2 fold greater than their respective control groups, supporting the results obtained in the in vitro model. The study thus shows that *Cannabis sativa* and the cannabinoids, THC and CBN, display anticoagulant activity and may be useful in the treatment of diseases such as type 2 diabetes in which a hypercoagulable state exists.

Keywords: Cannabis; Blood coagulation; Obesity; Anticoagulant; Cannabinoids

Introduction

The cannabinoids are the major active constituents of *Cannabis sativa.* Cannabinoids are oxygen-containing aromatic hydrocarbon compounds (Pertwee, 1999). There are about 70 known naturally occurring cannabinoids (Williamson and Evans, 2000). The three major cannabinoids are Cannabidiol (CBD), Cannabinol (CBN) and Tetrahydrocannabinol (THC) (Ashton, 2001). THC and its homologues have been found to be the main psychoactive constituents of Cannabis (Katzung, 1989).

Cannabis has been used as a therapeutic agent against numerous diseases for many years (Berry and Mechoulam, 2002). In South African indigenous medicine it is used to treat a wide range of conditions such as bronchitis, pain and diabetes (van Wyk and Gericke, 2000). The entire coagulation cascade is dysfunctional in diabetes. Both thrombosis and defective clot dissolution are favoured in diabetics (Vinik et al., 2001).

Insulin resistance (IR), which occurs in type 2 diabetes, appears to be a common precursor of both diabetes and macrovascular disease. Metabolic disturbances that commonly occur in patients with IR are atherogenic dyslipidemia, hypertension, glucose intolerance and a prothrombotic state. The prothrombotic state is characterized by increased fibrinogen levels, increased plasminogen activator inhibitor (PAI)-1 and different abnormalities in platelet function. Thrombosis is thus promoted and thrombolysis is retarded (Vinik et al., 2001).

Insulin resistance affects the procoagulant state by co-segregating with abnormalities involved in coagulation, including platelet aggregability, platelet adhesion and

*Corresponding author. Tel.: +27 41 504 4123; fax: +27 41 504 2814.
E-mail address: carminita.frost@nmmu.ac.za (C.L. Frost).

0944-7113/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2006.02.004

increased levels of thromboxane, von Willebrand factor, factor VIII, tissue plasminogen activator and fibrinogen. It decreases fibrinolytic activity due to increased levels of PAI-1. Levels of plasma insulin, proinsulin and glucose, and the concentration of modified lipoproteins all affect PAI-1 release. High levels of PAI-1 have been consistently associated with increased insulin concentrations and decreased insulin sensitivity (Vinik et al., 2001).

Circulating levels of free fatty acids are elevated in diabetics because of their excess liberation from adipose tissue and diminished uptake by skeletal muscle. The liver responds by increasing very-low-density lipoprotein production and cholesteryl ester synthesis resulting in hypertriglyceridemia (Creager et al., 2003). Hypertriglyceridemia may present a procoagulant state since PAI-1 is increased in the presence of hypertriglyceridemia (Tholstrup et al., 2003).

Since Cannabis has been used in South African indigenous medicine to treat diabetes and given the association of a procoagulant state with type 2 diabetes, we undertook an investigation to determine the effect of Cannabis and the three major cannabinoids, THC, CBD and CBN, on blood coagulation.

Materials and methods

Cannabis extract

The extract was prepared by following the cannabinoid extraction procedure described by Turner and Mahlberg (1984). 10 g of dried Cannabis was ground with a mortar and pestle. 10 ml of analytical grade chloroform was added and allowed to react for 1 h. The dried Cannabis was extracted three times and the fractions were combined and filtered using Millex Millipore filters (0.45 μm). The filtrate was evaporated under a gentle stream of nitrogen (on ice and protected from light). The remaining resin was resuspended in 2 ml of 100% methanol, flushed with nitrogen and stored under vacuum at 4 °C and protected from light (in an aluminium-covered container). THC (Industrial Analytical), CBD and CBN (Sigma) content was quantified using high performance liquid chromatography (HPLC) against known standards.

Thrombin assay

The thrombin assay involves the kinetic monitoring of the enzymatic action of thrombin on the synthetic substrate S2238. S2238 is composed of phenylalanyl-pipecolyl-arginine-ρ-nitroaniline-hydrochloride. Thrombin is specific for this substrate. The substrate is cleaved to yield a yellow product, the formation of which is measured spectrophotometrically at consecutive intervals at a wavelength of 412 nm (Liu et al., 1997). The procedure was carried out in a microtitre plate (Labsystems Multiskan MS) using the method specified by the substrate manufacturer, Chromogenix. Bovine thrombin was obtained from Sigma. The cannabis extract was tested at concentrations of 0–10 mg/ml. The three cannabinoids were tested at concentrations of 0–2 mg/ml.

Turbidimetric clotting assay

Radhakrishnamurthy et al. (1980) described a thrombin clotting assay performed in test tubes where the clotting time was measured by observing the formation of a fibrin thread. This method was modified to be performed in 96 well microtitre plates. Instead of observing the formation of the fibrin thread, the clotting reaction was monitored turbidimetrically by measuring the absorbance at 412 nm.

In vitro assay

Saline, extract or cannabinoid (30 μl) was added to 100 μl of human plasma. Clotting was initiated by the addition of 20 μl of 5 U/ml bovine thrombin (Sigma). The absorbance was monitored at 412 nm at 30 s intervals for 10 min.

In vivo assay

Obese rats were fed on a cafeteria diet for 8 weeks. Lean rats were fed on rat chow. Water was available *ad libitum*. Experimental rats were injected with cannabis extract subcutaneously every alternate day for 28 days. The first five treatments contained an equivalent of 5 mg/kg body weight THC. The remaining treatments contained an equivalent of 2.5 mg/kg body weight THC. The control groups were treated with 1% Tween 80 dissolved in saline.

At the end of the experiment blood was drawn from the rats and collected using a syringe containing 0.105 M sodium citrate. The blood was then centrifuged at 900 g for 15 min. The triglyceride, HDL, LDL and total cholesterol of the plasma was determined and the atherogenic index (AI) of pooled plasma from each group of rats was calculated (AI = (total cholesterol–HDL cholesterol)/HDL cholesterol). The plasma collected was stored at −20 °C until the clotting assay was conducted. The rat plasma was assayed in a similar manner as the human plasma.