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Momordica charantia extract, a herbal remedy for type 2 diabetes, contains a specific 11β-hydroxysteroid dehydrogenase type 1 inhibitor

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ABSTRACT

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) catalyzes the intracellular regeneration of active cortisol from inert cortisone in key metabolic tissues, thus regulating ligand access to glucocorticoid receptors. There is strong evidence that increased adipose 11β-HSD1 activity may be an important aetiological factor in the current obesity and diabetes type 2 epidemics. Hence, inhibition of 11β-HSD1 has emerged as a promising anti-diabetic strategy, a concept that is largely supported by numerous studies in rodent models as well as limited clinical data with prototype inhibitors. *Momordica charantia* (also known as bitter melon, bitter gourd or karela) is traditionally used for treatment of diabetes in Asia, South America, the Caribbean, and East Africa. In the present study, we show that *M. charantia* extract capsules contain at least one ingredient with selective 11β-HSD1 inhibitory activity. The finding constitutes an interesting additional explanation for the well-documented anti-diabetic and hypoglycaemic effects of *M. charantia*.

1. Introduction

Type 2 diabetes is a major global health problem, currently affecting more than 200 million people worldwide [1], a number projected to rise to over 300 million in 2030 [2]. The disease causes substantial morbidity, mortality, and long-term complications and represents an important risk factor for cardiovascular disease. Therefore, the need for new therapy concepts to treat this metabolic disorder is apparent.

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) is a microsomal enzyme that converts glucocorticoid receptor (GR)-inert cortisone (dehydrocorticosterone in rodents) to active cortisol (corticosterone in rodents) and thus acts as an intracellular switch to mediate glucocorticoid action in metabolic tissues [3,4]. During the last decade, multiple evidence has accumulated that argues for an aetiological role of 11β-HSD1 in obesity and type 2 diabetes [5–7]. 11β-HSD1-deficient mice show enhanced glucose tolerance, improved hepatic insulin resistance, attenuated gluconeogenesis, and an improved lipid and lipoprotein profile [8–10]. Moreover, transgenic overexpression of 11β-HSD1 selectively in adipose tissue causes visceral obesity, insulin resistance, type 2 diabetes, dyslipidemia, and hypertension in mice [5]. Collectively, these findings emphasize the potential benefits of a specific inhibitor of human 11β-HSD1 in the treatment of obesity and type 2 diabetes. An important requirement for this inhibitor is selectivity, *i.e.* it must not equally inhibit 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), the enzyme catalyzing the reverse reaction in mineralocorticoid target tissues, as this leads to severe side effects such as renal sodium retention, hypokalaemia and hypertension [11,12].

Momordica charantia (also known as bitter melon or bitter gourd) belongs to the Cucurbitaceae family and has a long history of use as a hypoglycaemic agent amongst the indigenous populations of Asia, South America, the Caribbean, and East Africa [13,14]. Numerous animal studies support its anti-diabetic effects, including improvement of glucose tolerance, enhancement of lipolysis as well as protection from insulin resistance and diet-induced adiposity [15–23]. But even though numerous studies have addressed the pharmacological properties of *M. charantia*, neither the hypoglycaemically active ingredients nor the underlying mode of action can be considered well-characterized. With the potential role of 11β-HSD1 in the aetiology of diabetes type 2 in mind, we sought to assess whether commercially available *M. charantia* extract capsules contained an 11β-HSD1 inhibitor. To this end, *M. charantia* extracts were tested for inhibition of 11β-HSD1 and 11β-HSD2 *in vitro*, with the result that they contain at least one specific 11β-HSD1 inhibitor.

Abbreviations: GR, glucocorticoid receptor; 11β-HSD, 11β-hydroxysteroid dehydrogenase; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; 11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2; IRS1, insulin receptor substrate 1.

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2. Material and methods

2.1. Processing of M. charantia extract

Capsules containing *M. charantia* extract (one capsule containing 0.38 g dry weight, Sanakine, Schmidt GmbH, Germany; formerly Glukokine, Sandoz) were purchased from the local pharmacy. The capsules were cut open with a surgical blade, the paste-like content was washed three times with 5 ml n-hexane and dried. The residue was resuspended in DMSO (approximately 0.5 ml/capsule), insoluble material was removed by centrifugation and the resulting supernatant was used directly in enzyme inhibition experiments with subsequent detection by HPLC or stored at −20 °C.

For fluorescence detection the extract was diluted one to one with water to yield a DMSO concentration of 50%.

2.2. Preparation of 11β-HSD1-containing microsomes and purification of human 11β-HSD1

Human liver microsomes and purified human 11β-HSD1 were prepared as described previously [4,24].

2.3. Preparation of 11β-HSD2-containing microsomes from placenta

Human placental microsomes containing 11β-HSD2 were prepared as follows: The placenta samples were homogenized in 4 volumes of homogenization buffer (20 mM Tris/HCl, 250 mM sucrose, 1 mM EDTA, 0.1 mM PMSF, pH 7.4) using a glass-Teflon Potter-Elvehjem homogenizer. The homogenate was centrifuged at 600 × *g* for 10 min followed by 10,000 × *g* for 10 min to sediment nuclei, cell debris, and mitochondria. The resulting supernatant was centrifuged at 170,000 × *g* for 1 h to sediment the microsomes. The microsomal pellet was resuspended in the homogenization buffer finally yielding a protein concentration of about 20 mg/ml.

2.4. Enzymatic assays

For analysis of inhibition of 11β-HSD1 reductase activity the conversion of cortisone to cortisol was measured as follows: 10 μl purified human 11β-HSD1 or 10 μl of microsomes containing human 11β-HSD1 were added to 50 μl 5 mM cortisone solution (final concentration: 500 μM) and 20 μl NADPH-regenerating system in 10 mM phosphate buffer pH 7.4 (final assay concentrations $NADP^+$ 0.5 mM; glucose-6-phosphate 3.5 mM; glucose-6-phosphate dehydrogenase 5 μg/ml; $MgCl_2$ 2 mM). After addition of the *M. charantia* extracts (5–50 μl) 20 mM phosphate buffer pH 7.4 was added to the reaction mixture to yield a final assay volume of 500 μl. The mixture was incubated for 3 h at 37 °C. 11β-HSD activities were not significantly affected by DMSO concentrations up to 10%.

For analysis of inhibition of 11β-HSD1 dehydrogenase activity the oxidation of cortisol to cortisone was measured essentially as above, except for the substitution of the regenerating system with 2.5 mM $NADP^+$ only, and the use of 500 μM cortisol instead of cortisone. Here, the incubation time was 60 min. For analysis of inhibition of 11β-HSD2 dehydrogenase activity the assay performed was again essentially the same, except for the use of 2.5 mM NAD^+ as cofactor and of human placental microsomes containing 11β-HSD2.

After incubation the reaction was stopped by adding 250 μl ice-cold ethylacetate and vortexing, followed by centrifugation. Then, the two phases were separated and the aqueous phase was shaken out two more times with 250 μl ethylacetate. The ethylacetate phases were pooled and evaporated in a SpeedVac (ThermoSavant). The resulting residue was dissolved in 50 μl methanol-H_2O (58:42, v/v) and 10 μl-aliquots were used for metabolite determination by HPLC.

2.5. Metabolite determination by HPLC

After enzymatic conversion the glucocorticoids were resolved on a Merck reversed phase HPLC system, with a LiChrospher 100 RP-18 (5 μm) column, 4.5 × 25 mm. Methanol–H_2O (58:42, v/v) was used as the mobile phase with a flow rate of 0.5 ml/min. Absorbance of the glucocorticoids was monitored at 262 nm. Product yield was quantified by peak integration of the cortisone and cortisol peaks, respectively.

2.6. Cortisol determination by homogenous time resolved fluorescence (HTRF)

Inhibition of 11β-HSD1-catalyzed cortisone reduction with *M. charantia* extract was measured by HTRF using a cortisol detection assay from Cisbio. The kit was used according to the manufacturer's instructions. The assay was incubated for 2 h at 37 °C in a total volume of 50 μl (10 μl [4.7 μg] purified enzyme, 10 μl *M. charantia* extract, 15 μl 200 mM phosphate buffer pH 7.4, 3 μl 3.33 mM NADPH, 3 μl 2.67 μM cortisone and 9 μl water) in a black half-area 96-well plate (Costar). Then, 25 μl d2-conjugate and 25 μl cryptate-conjugate were added and the mixture was incubated for another 2 h at room temperature. HTRF signals were read on a microplate reader (Genios Pro, Tecan) with the following settings:

Measurement 1: excitation filter, 320 nm; emission filter, 620 nm; mirror, dichroic3; lag time, 150 μs; integration time, 500 μs; number of flashes, 10; optimal gain; optimal z-pos.

Measurement 2: excitation filter, 320 nm; emission filter, 665 nm; mirror, dichroic3; lag time, 150 μs; integration time, 500 μs; number of flashes, 10; optimal gain.

3. Results

Inhibition of 11β-HSD1 and 11β-HSD2 activities was assessed by determining cortisone (oxidative reaction) or cortisol (reductive reaction) yields after incubation with various volumes of *M. charantia* extract. Quantification was performed by integration of the well-resolved product peaks in the HPLC chromatogram where the retention times obtained were 13.5 min for cortisone and 16.75 min for cortisol (Fig. 1). Product yields of the controls without *M. charantia* extract were normalized to 100% enzyme activity and the residual activity expressed as percent of the control (Fig. 2). Also, inhibition of 11β-HSD1-catalyzed reduction of cortisone was monitored by homogeneous time-resolved fluorescence (Fig. 3). The results show that addition of *M. charantia* extract leads to a dose-dependent decrease of cortisol yield when 11β-HSD1-mediated cortisone reduction was followed (Figures 1A, 2A and 3). Here, an IC50 value of log −2.58 ± 0.077 g/ml was determined for *M. charantia* extract (Fig. 3). The same pattern was observed when the reverse reaction direction was assessed (Figs. 1B and 2B), whereby inhibition of the dehydrogenase reaction appeared to be somewhat more efficient (Fig. 2A and B). In contrast, no changes in product yield and thus no changes in activity were observed when 11β-HSD2-mediated oxidation of cortisol was assessed (Fig. 2C). In summary, incubation of human 11β-HSD1 with *M. charantia* extract *in vitro* resulted in a dose-dependent and selective inhibition of 11β-HSD1 activity (Figs. 1 and 2).

4. Discussion

For a long time, *M. charantia* has been popular as traditional medicine in the treatment of diabetes in Asia, South America, the

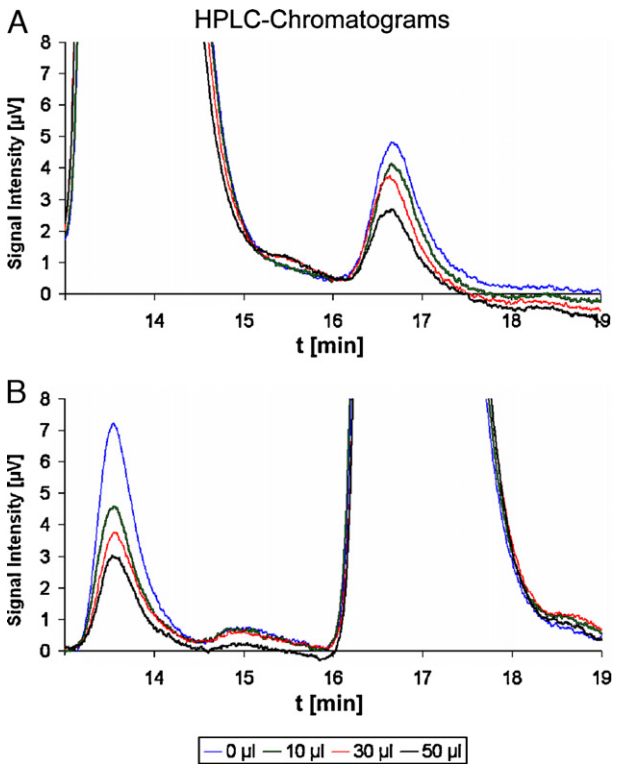

Fig. 1. HPLC derived product peaks after 11β-HSD1-catalyzed cortisone reduction and cortisol oxidation. Representative HPLC chromatogram sections show the cortisone (retention time 13.5 min) and cortisol (retention time 16.75 min) peaks after 11β-HSD1-mediated conversion in presence of different volumes of *M. charantia* extract. For clarity reasons, only the results using 0 µl, 10 µl, 30 µl and 50 µl *M. charantia* extracts are shown. **A**. HPLC chromatograms after 11β-HSD1-mediated reduction of cortisone, showing decreasing cortisol peak areas in response to increasing volumes of *M. charantia* extract. **B**. HPLC chromatograms after 11β-HSD1-mediated oxidation of cortisol, showing decreasing cortisone peak areas in response to increasing volumes of *M. charantia* extract. In contrast, *M. charantia* extract had no effect on human 11β-HSD2 dehydrogenase activity (data not shown).

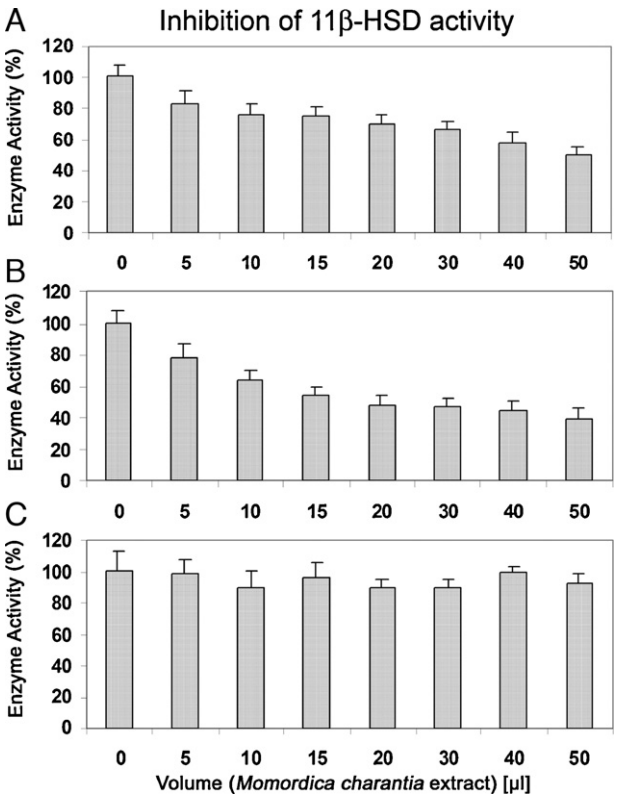

Fig. 2. Inhibition of microsomal 11β-HSD1 activity by *M. charantia* extracts. Product yields were quantified by integration of cortisol or cortisone peaks in the HPLC chromatograms (see Fig. 1). The product yields of the controls without *M. charantia* extract were normalized to 100% enzyme activity and the residual activity expressed as percent of the control. **A**. Inhibition of 11β-HSD1 reductase activity by various amounts of *M. charantia* extracts. **B**. Inhibition of 11β-HSD1 dehydrogenase activity by various amounts of *M. charantia* extracts. **C**. 11β-HSD2 dehydrogenase activity in presence of various amounts of *M. charantia* extracts. The results are presented as means ± SD from five experiments.

Caribbean, and East Africa. Nowadays, it is sold as a food supplement all over the world to help lower glucose intolerance and control hyperglycaemia. The presented findings indicate the presence of a selective 11β-HSD1 inhibitor in *M. charantia* extract.

Multiple mechanisms have been proposed to explain *M. charantia*'s hypoglycaemic properties [25]. Early investigators suggested a (hitherto unidentified) insulin-like hormone peptide as the active ingredient [26–28], which agrees well with observations of insulinomimetic effects, particularly in terms of decreased gluconeogenesis, increased glucose uptake and glycogen synthesis in liver and muscle [17,19,29–34]. Others proposed an inhibitor of intestinal glucose absorption [35–37] or a compound that enhances insulin secretion from the pancreatic β-cells by regenerating pathologically altered islets of Langerhans and/or by acting as an insulin secretagogue [38–40]. More recent findings suggest the presence of an activator of AMP-activated kinase, a master regulator of energy metabolism [41], but also of compounds that stimulate *PPARA* and *PPARG* gene expression, both genes encoding pivotal ligand-activated transcription factors in the regulation of lipid metabolism [42]. Also three structurally related PPARα agonists have been identified in *M. charantia*, but remain to be studied in terms of hypoglycaemic effects [43]. Furthermore, there is evidence that *M. charantia* juice can protect high-fat-fed rats from insulin resistance by activating insulin receptor substrate 1 (IRS1) in skeletal muscle by tyrosine phosphorylation, a highly interesting observation in the context of type 2 diabetes [44].

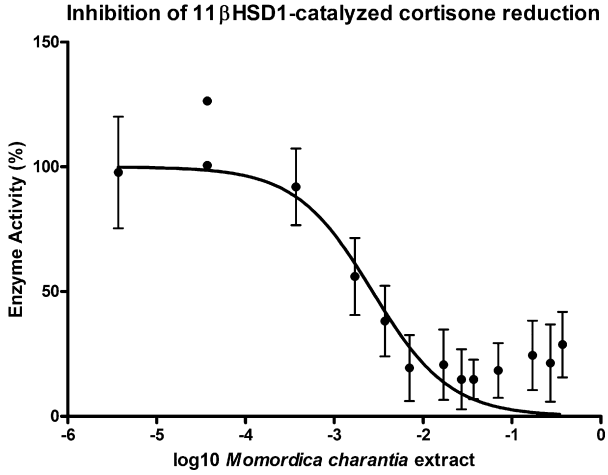

Fig. 3. Inhibition of purified 11β-HSD1 activity by *M. charantia* extracts. The product yields of the controls without *M. charantia* extract were normalized to 100% enzyme activity and the residual activity expressed as percent of the control. Inhibition of 11β-HSD1 reductase activity by various amounts of *M. charantia* extracts was measured by HTRF as detailed in the methods section. Results are presented as means ± SD (n = 5–7) or as single values (n = 2), respectively. Data were analyzed by non-linear regression (GraphPad Prism, v. 4.03, GraphPad Software) using a sigmoidal dose-response equation with top = 100% and bottom = 0% resulting in an EC50 of 2.63 ± 0.5 g/ml. The deviation of the data points from the calculated curve at high *M. charantia* extract concentrations are probably the result of a beginning precipitation of poorly soluble components.

To our knowledge, only one study describes the isolation of two pure and hypoglycaemically active compounds, notably both with a triterpene/steroid scaffold and thus potential inhibitors of 11β-HSD1 [45]. Remarkably, some of the *M. charantia*-mediated pharmacological effects described above are highly reminiscent of consequences of 11β-HSD1 inhibition: First, selective and non-selective 11β-HSD1 inhibitors can stimulate insulin secretion in pancreatic islets [46–48]. Second, as expression of the rate-limiting gluconeogenic enzyme, phosphoenolpyruvate carboxykinase, is GR-dependent and thus controlled by intracellular cortisol concentrations, inhibition of 11β-HSD1 attenuates hepatic gluconeogenesis [49,50]. Third, a selective 11β-HSD1 inhibitor has been shown to abolish inactivation of IRS1 by serine phosphorylation in skeletal muscle which otherwise leads to disruption of the insulin signalling cascade [51].

However, certainly inhibition of 11β-HSD1 cannot explain the whole range of *M. charantia*-mediated hypoglycaemic effects described in the literature and some observations are even contradictory. For instance, *PPARA* is a glucocorticoid-responsive gene and as such subject to modulations by intracellular cortisol concentrations regulated via 11β-HSD1. Inhibition of 11β-HSD1 should decrease *PPARA* gene expression, but the opposite is observed upon supplementation with *M. charantia* extract. Furthermore, it should be critically noted that the test systems used in the presented study, *i.e*. the purified enzyme or microsomal preparations, neither take into account intracellular metabolism of active *M. charantia* ingredients nor whether they can penetrate cell membranes in relevant metabolic target tissues.

Nonetheless, it is conceivable that inhibition of 11β-HSD1 contributes significantly to the overall hypoglycaemic action of *M. charantia*. Considering the potential benefits of a selective 11β-HSD1 inhibitor for treatment of obesity and type 2 diabetes, the identification and characterization of the indicated active ingredient(s) is clearly of high interest and the *in vitro* test systems described in this study are suitable to assess isolated compounds.

In conclusion, we report that *M. charantia* extract contains a selective 11β-HSD1 inhibitor. The finding provides an additional molecular mechanism for the widely recognized anti-diabetic effects of *M. charantia*, which most likely is the result of a concerted effect involving several active ingredients.

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