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Emodin, a compound with putative antidiabetic potential, deteriorates glucose tolerance in rodents

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ABSTRACT

Emodin is found in remedies from Traditional Chinese Medicine. Since antihyperglycaemic action was observed in rodents, non-scientific sources advertise emodin intake as a natural cure for diabetes. Emodin was admixed to high fat-food of obese mice at two doses (2 and 5 g/kg; daily emodin uptake 103 and 229 mg/kg). Comparison was made to ad libitum fed and to food restricted control groups, the latter showing the same weight gain as the corresponding emodin-treated groups. Emodin blunted food intake by 6% and 20% for the low and high dose, which was accompanied by proportionate reductions in weight gain. Emodin reduced blood glucose relative to freely feeding controls, but comparison to weight-matched controls unmasked deterioration, rather than improvement, of basal glycaemia (mmol/l: fed ad libitum, 9.5 ± 0.4; low emodin, 9.4 ± 0.3, weight-matched, 8.2 ± 0.3; high emodin, 7.2 ± 0.4, weight-matched, 6.1 ± 0.3; $P < 0.01$, emodin vs weight-matched) and glucose tolerance (area under the curve, min*mol/l: fed ad libitum, 2.01 ± 0.08; low emodin, 1.97 ± 0.12, weight-matched, 1.75 ± 0.03; high emodin, 1.89 ± 0.07, weight-matched, 1.65 ± 0.05; $P < 0.0002$, emodin vs weight-matched). An insulin tolerance test suggested insulin desensitisation by prolonged emodin treatment. Furthermore, a single oral emodin dose did not affect glucose tolerance in obese mice, whereas intravenous injection in rats suggested a potential of emodin to acutely impair insulin release. Our results show that the antihyperglycaemic action of emodin as well as associated biochemical alterations could be the mere consequences of a spoilt appetite. Published claims of antidiabetic potential via other mechanisms evoke the danger of misuse of natural remedies by diabetic patients.

1. Introduction

In Traditional Chinese Medicine, numerous remedies derived from plants and animals are used for the treatment of type 2 diabetes mellitus. The anthraquinone derivative emodin (1,3,8-trihydroxy-6-methylanthra-9,10-quinone), which is found in roots and barks of many plants, is among the putative active ingredients believed to account for antidiabetic effects (Li et al., 2004; Xie and Du, 2011). Under academic examination, emodin exhibited a broad pharmacology with promising evidence for laxative, antiinflammatory, anticancer and other beneficial activities (Shrimali et al., 2013; Srinivas et al., 2007), which included the lowering of blood glucose in hyperglycaemic rodents (Feng et al., 2010; Wang et al., 2012; Xue et al., 2010; Zhao et al., 2009). Although the evidence is purely experimental, antihyperglycaemic action in rodents is meanwhile extensively cited by vendors to promote emodin-containing herbal products as a natural cure for diabetes.

Studies aiming to understand the mechanism(s) responsible for emodin-induced lowering of blood glucose gave rise to a surprising multitude of suggested molecular targets and alleged biochemical mechanisms. Some reports proposed that emodin acts via mechanisms attributed to clinically established antidiabetic drugs, providing evidence for a thiazolidinedione-like mode of action by binding to peroxisome proliferator-activated receptor-γ (PPARγ) and promotion of adipocyte differentiation (Chen et al., 2012; Yang et al., 2007), as well as for a metformin-like mode of action via inhibition of mitochon-

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drial complex 1, impairment of cell respiration and activation of AMP-activated protein kinase (AMPK) (Song et al., 2013). Again other studies attributed emodin's antihyperglycaemic activity to modulation of the sterol regulatory element binding protein (SREBP) pathway (Li et al., 2016) or to direct interaction with the enzymes 11β-hydroxysteroid dehydrogenase (11β-HSD) (Feng et al., 2010; Wang et al., 2012), glycogen synthase kinase-3β (GSK-3β) (Gebhardt et al., 2010), or acetyl-CoA carboxylase (ACC) (Chao et al., 2010). And finally, improvement of glucose homeostasis has also been related to effects on pancreatic β-cells, in which emodin was claimed to upregulate L-type calcium channels that are essential mediators of insulin release (Zhao et al., 2009).

In the light of such versatile information about potential molecular targets and pathways, it is puzzling how little efforts have been made to evaluate the straightforward possibility that improvements in insulin sensitivity and glucose homeostasis could result from reductions in appetite and body weight, as they have also been observed in emodin-treated rodents (Feng et al., 2010; Li et al., 2016; Lu et al., 2014; Wang et al., 2012). This question has been addressed in the present study, which revealed that the beneficial effects of subchronic emodin treatment on glucose homeostasis are entirely dependent on blunted appetite and weight gain. Our results even unmask that emodin causes deterioration rather than improvement of glucose tolerance, when comparison is made to restrictedly fed, weight matched control animals.

2. Material and methods

2.1. Chemicals

Emodin was purchased from FWD Fine Chemicals Limited (Shanghai, China). Before use, 98% purity as stated by the supplier was confirmed in-house by nuclear magnetic resonance.

2.2. Animal husbandry

Six weeks-old male C57BL/6J mice were purchased from Charles River Laboratories, Sulzfeld, Germany. Mice were housed in polycarbonate cages provided with wood-based bedding (Hygienic Animal Bedding, J.Rettenmaier & Söhne, Rosenberg, Germany), under constant room temperature and with an artificial 12 h dark/12 h light cycle. Unless stated otherwise, mice had free access to tap water and high fat diet (HFD; 60% of calories as fat; diet D12492 from Research Diets Inc., New Brunswick, NJ, USA).

Male Sprague-Dawley rats were from the breeding facilities of the Division for Laboratory Animal Science and Genetics, Medical University of Vienna (Himberg, Austria) and were used at a body weight of approximately 400 g. Husbandry was as described for mice, except that rats were fed a conventional rodent chow diet (sniff R/M-H; sniff Spezialdiäten GmbH; Soest, Germany).

The study was in line with effective national and international guidelines and law, and all procedures followed the principles of good laboratory animal care. The protocol was approved by the Austrian Federal Ministry of Science, Research and Economy.

2.3. Effects of a single emodin dose in obese mice

At an age of 15 weeks and after 9 weeks of HFD feeding, 36 mice were allocated to 3 wt-matched groups (n=12 each). They were fasted for 10 h before the tip of the tail was pricked with a needle for the measurement of blood glucose with a portable glucose meter (OneTouch, LifeScan, Milpitas, CA, USA; means of duplicate measurements). Immediately thereafter, mice received by gavage a suspension of 100 or 250 mg/kg emodin, or the vehicle (0.5% sodium carboxymethyl cellulose; 5 µl/g body weight) with the doses corresponding to daily oral doses used for repeated treatment in the present study as well as in an earlier study (Feng et al., 2010). Thirty min after dosing, an intraperitoneal glucose tolerance test (IPGTT) was started. Mice were injected with glucose solution (33% wt/vol; 1 g/kg) and the resulting excursion of blood glucose was documented by measurements immediately before (0 min) as well as 20, 40, 60, 90, and 120 min after glucose administration.

To assure that relevant actions are not missed due to parenteral glucose administration or due to a slow onset of emodin action, oral glucose tolerance tests (OGTTs) were performed. After 9 weeks on HFD 10 obese mice were fasted 10 h and fed 250 mg/kg emodin as described above. Two oral doses of a 33% (w/v) glucose solution (2 g/kg) were administered by gavage 30 min and 240 min after administration of emodin. The experiment was performed in two runs that were one week apart. In each run, half of the mice received emodin and vehicle, providing a paired data set.

2.4. Effects of subchronic emodin treatment in obese mice

After 10 weeks of HFD feeding, one mouse out of 60 was excluded for its disproportionally low body weight. The 59 remaining mice were divided into 5 wt-matched groups to study the effects of subchronic emodin treatment. One group continued on HFD ad libitum (referred to as freely feeding controls; n=11); two groups had free access to HFD with either 2 or 5 g/kg emodin admixed (referred to as low and high dose emodin; n=12 each); and two groups were subjected to restricted feeding with HFD, aiming at rates of weight gain as seen in the low and high dose emodin groups (referred to as food restricted controls; n=12 each). The food admixtures of 2 and 5 g/kg emodin resulted in average daily intake of 103 and 229 mg/kg emodin, which resembles the doses previously associated with antihyperglycaemic action (Feng et al., 2010). Food intake and body weight were documented every 3–4 days. Furthermore, immediately before as well as 24 and 51 days after starting the treatment, fat mass and lean mass of each mouse were determined by nuclear magnetic resonance (EchoMRI, Houston, TX, USA).

Mice on low dose emodin, their corresponding food restricted controls, and half of the freely fed controls were examined in an IPGTT on days 21 and 48 of treatment. High dose emodin mice, their food restricted controls, and the remaining freely fed controls were tested one day later (days 22 and 49). Mice were fasted for 10 h and basal blood glucose was measured. Twenty min later, mice were injected with glucose to start the IPGTT. Detailed procedures were as in the IPGTT after administration of a single emodin dose (see chapter 2.3).

On day 29 of emodin treatment, mice on high dose emodin, their food restricted controls, and the freely fed controls were subjected to an insulin tolerance test (ITT). Food was withdrawn 1 h before blood glucose was measured and 0.75 U/kg insulin was injected intraperitoneally (NovoRapid, Novo Nordisk, Bagsvaerd, Denmark, diluted with saline; injected volume, 3 µl/g). Further measurements of blood glucose were made 15, 30, 45, and 60 min after insulin injection. In mice that showed a blood glucose value < 2.2 mmol/l, the ITT was immediately terminated by an intraperitoneal injection of 33% glucose solution.

On day 56 of treatment, mice were killed in the fed state with an overdose of an inhalation anaesthetic (Sevoflurane, Sigma-Aldrich, St. Louis, MO, USA). Blood was collected by heart puncture and plasma was stored at −20 °C for the later measurement of plasma insulin (Ultrasensitive Mouse Insulin ELISA from Mercodia, Uppsala, Sweden) and of plasma emodin. Emodin was measured by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) from plasma samples of 100 µl after spiking with aloe emodin (used as internal standard), followed by extraction and reconstitution to a volume of 500 µl. Recovery of the internal standard was 1.0 ± 0.1 (mean ± S.D.). Procedures are outlined in further detail in Supplementary file S1.