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Identification of PPARγ agonists in extracts of carrots (Daucus carota) by bioassay-guided fractionation

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Introduction
One of the major characteristics of type 2 diabetes (T2D) is insulin resistance, which is often treated by insulin sensitizing drugs such as thiazolidinediones (TZDs). The primary target for the TZDs is the peroxisome proliferator-activated receptor (PPARγ). However, critical side effects of TZDs can occur, as they are full PPARγ agonists. Partial PPARγ agonists are associated with fewer side effects but still may maintain the effect on insulin resistance.

Carrots are known to contain highly bioactive aliphatic polyacetylenes, which are also present in related vegetables of the Apiaceae family [1]. These polyacetylenes are very similar in chemical structure to natural ligands for the PPARγ, such as fatty acids, and hence may have important PPARγ activating properties.

Plant extract
10 kg carrots (cv. Bolero and cv. Purple Haze) were macerated and then extracted by a 2-step sequential extraction procedure using dichloromethane (DCM) and methanol (MeOH). The extraction mixture was allowed to stand for 24 hours in the dark at 5 °C before filtering and re-extraction. Extracts were dried under vacuum and then re-dissolved in DMSO for testing.

Transactivation assay
The ability of the extract to activate the PPARs (α,β and γ) was tested in a luciferase-based PPAR transactivation assay using mouse fibroblast cells transfected with a luciferase reporter plasmid, a transfection control plasmid, and an expression plasmid. Degree of activation was determined by a luminometer and compared to a positive control, Rosiglitazone (Rosi). The DCM extract of both varieties were found to activate PPARγ in a dose dependent manner (1-100 μg/mL) (Fig. 1) and subjected to bioassay-guided fractionation (data not shown).

Stimulation of glucose uptake in adipocytes
Mature adipocytes were subjected to the extracts and after 2 days glucose-uptake were induced by insulin. Effect was measured using 14C-labeled glucose and afterwards radioactivity was determined by a scintillation counter. All results were compared to a positive control (Rosi) and vehicle (DMSO) (Fig. 3).

Conclusion
The results clearly indicate that carrots contain partial PPARγ agonists with a potential in relation to the management of insulin resistance and T2D. Bioassay-guided fractionation showed that aliphatic C17-polyacetylenes such as falcarinol are responsible for the PPARγ activating properties of carrots (data not shown). The polyacetylenes warrant further investigation in order to identify the most promising PPARγ agonists in carrots.

References

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Fig. 1
Fold activation of PPARγ

Fig. 2
DMSO Rosi DCM extract 100 μg/mL

Fig. 3
Fold activation of PPARγ

nM insulin