Macedonian pharmaceutical bulletin, 68 (Suppl 2) 157 - 158 (2022)
ISSN: 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2022.68.04.071
Short communication

Orlistat decreases lipid accumulation and affects expression of genes related to lipid metabolism in *Caenorhabditis elegans*

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Introduction

Obesity is one of the leading causes for cardiovascular diseases, certain types of cancer, diabetes and reproductive failure (Loos and Yeo, 2022). There are currently few medications approved for obesity, while most people still rely on exercise and diet for weight control (Aaseth et al., 2021).

In recent years *C. elegans* has become a reasonable and easily manipulated model organism for obesity research due to the numerous advantages such as completely sequenced genome, short-life span, affordable mutant strains, 65% similarity to the human genome and conserved pathways for fat synthesis and lipolysis (Shen et al., 2018).

Orlistat is one of the drugs prescribed for obesity management, which acts as a pancreatic lipase inhibitor and reduces fat absorption in the intestine, although side effects of the treatment include gastro-intestinal dysfunction and diarrhea (Aaseth et al., 2021).

In order to study the molecular mechanisms associated with the treatment of orlistat we employed the *in vivo* system of *C. elegans* in high-glucose and non-glucose diet by applying lipid staining techniques, quantification of triglycerides and RT-qPCR for specific mRNAs and miRNAs associated with lipid metabolism.

Materials and methods

C. elegans maintenance and treatment

The wild type N2 Bristol was purchased by the Caenorhabditis Genetic Centre. The nematodes were grown at 20 °C according to standard procedures on Nematode Growth Medium (NGM) plates seeded with *E. coli* strain OP50 as a food source.

For the following experiments, standard hypochlorite bleaching was used to obtain age-synchronized worms. Nematodes were maintained on NGM plates (3000-4000 worms per plate) with glucose (2%). Normal *E. coli* OP50 was used as a food source until reaching L2 phase. Then worms were transferred to new NGM plates with glucose, previously treated with Orlistat (12 μm) and seeded with heat-inactivated *E. coli* OP50 as a food source.

The nematodes were treated for 24 hours, until they reach L4 larvae stage, then they were collected with M9 buffer and used for following assays.

Oil red O staining

Lipid staining assay was performed as previously described (Wang et al., 2021). The worms (around 1000-1500 per plate) were collected and washed with M9 buffer,

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followed by fixation in 60% solution of isopropanol for 5 min. Oil red O was added to each sample and incubated for 6 hours at room temperature. The quantification of the stained lipid droplets was measured as an average pixel intensity using Image J software.

Nile red staining

Around 1000-1500 nematodes were fixed with 40% isopropanol for 3 mins at RT (room temperature), then stained according to (Stuhr et al., 2022). The nematodes were imaged on confocal system Leica Stellaris 5 with inverted microscope Leica dmi8. Quantification of fluorescence density was performed *via* Image J software.

Triglyceride quantification

The quantification of triglycerides was performed following the manufacturer instructions of Triglyceride Quantification kit (MAK266, MilliporeSigma).

Quantative real-time polymerase chain reaction (RT-qPCR)

Total RNA was extracted via PureZol (Bio-Rad) from 3000-4000 nematodes per group. Reverse transcription for mRNAs was performed using First strand cDNA synthesis kit (Canvax) and for microRNAs Revert Aid H Minus First Strand cDNA Synthesis kit (ThermoFisher) with the use of stem-loop primers (Chen et al., 2005). Expression of mRNAs and microRNAs associated with lipolysis and lipogenesis were quantified by $_{\Delta\Delta}$CT method on the CFX Maestro software (Bio-Rad). *Iscu-1* and *mdh-11* were used for endogenous control for mRNAs and *u18* was used for reference gene for the miRNAs as well as the exogenous control *ath-miR-159a*.

Results and discussion

Our results show that orlistat decreases lipid accumulation in both non-glucose and glucose supplemented groups. Glucose supplementation upregulated *atgl-2*, *hlh-11*, *nhr-49*, *fasn-1* and *pod-2*, while *lipl-3* was downregulated compared to the non-glucose group. Relative mRNA expression of *acs-2*, *atgl-2*, *hlh-11*, *pod-2*, *fasn-1* and *nhr-49* was decreased in high-glucose orlistat treated group, compared to vehicle.

We performed screening of microRNAs associated with lipid metabolism and differential expression of miR-34 and miR-2 was observed in the non-glucose orlistat treated group compared to the vehicle.

Conclusion

Our findings confirm lipid-reducing effect of orlistat independently of glucose supplementation. Results from RT-qPCR of selected mRNAs and microRNAs suggest possible broader mechanism than lipase inhibition by orlistat.

Acknowledgements

This project for establishment of CPSBB has received funding from the European Union's Horizon 2020 research and innovation programme, project PlantaSYST (SGA No 739582 under FPA No. 664620), and the BG05M2OP001-1.003-001-C01 project, financed by the European Regional Development Fund through the "Science and Education for Smart Growth" Operational Programme.

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