

The Phytochemical Glaucarubinone Promotes Mitochondrial Metabolism, Reduces Body Fat, and Extends Lifespan of *Caenorhabditis elegans*

Authors

K. Zarse¹, A. Bossecker¹, L. Müller-Kuhrt², K. Siems², M. A. Hernandez³, W. G. Berendsohn⁴, M. Birringer¹, M. Ristow^{1,5}

Affiliations

Affiliation addresses are listed at the end of the article

Key words

- obesity
- aging
- lifespan
- phytochemicals
- mitochondria
- mitohormesis

Abstract

Naturally occurring compounds that promote energy expenditure and delay aging in model organisms may be of significant interest, since these substances potentially provide pharmaceutical approaches to tackle obesity and promote healthy lifespan in humans. We aimed to test whether pharmaceutical concentrations of glaucarubinone, a cytotoxic and antimalarial quassinoid known from different species of the plant family *Simaroubaceae*, are capable of affecting metabolism and/or extending lifespan in a nematodal model organism for aging processes, the roundworm *Caenorhabditis elegans*. Adult *C. elegans* roundworms, maintained on agar plates, were fed with *E. coli* strain OP50

bacteria, and glaucarubinone was applied to the agar to test (i) whether it alters respiration rates and mitochondrial activity, (ii) whether it affects body fat content, and (iii) whether it may promote longevity by quantifying survival in the presence and absence of the compound. We have found that glaucarubinone induces oxygen consumption and reduces body fat content of *C. elegans*. Moreover and consistent with the concept of mitohormesis, glaucarubinone extends *C. elegans* lifespan when applied at a concentration of 1 or 10 nanomolar. Taken together, glaucarubinone is capable of reducing body fat and promoting longevity in *C. elegans*, tentatively suggesting that this compound may promote metabolic health and lifespan in mammals and possibly humans.

Introduction

Promotion of longevity and in particular extension of healthy lifespan (also named 'healthspan') is of eminent interest to most humans. Specific mutations have been shown to extend the lifespan of model organisms dramatically [1–7], while more readily available interventions, including calorie restriction, extend life expectancy of model organisms, however, less strikingly [8,9]. Accordingly, considerable effort has been invested to identify naturally occurring and/or pharmaceutical compounds that promote longevity in model organisms. A number of such compounds have been identified in recent years, including rapamycin [10–12], resveratrol [12–16], and 2-deoxy-D-glucose [17].

Glaucarubinone is a quassinoid known to occur in *simaroubaceae* plants. This compound has been previously shown to exert cytotoxic [18–20] and antimalarial [21–23] properties; the exact mechanisms of action are, however, unknown [24]. We have now tested whether glaucarubinone at pharmaceutical doses may be effective in

reducing body fat and/or extending lifespan of a metazoal model organism, *C. elegans*.

Materials and Methods

Compound

Glaucarubinone was obtained from AnalytiCon Discovery, Potsdam, Germany. The compound was isolated from the stems of *Simarouba glauca*, collected in El Salvador. The structure was confirmed by NMR and LCMS spectra. The purity of the compound was >90% (determined by ¹H-NMR spectroscopy).

Maintenance and analyses of nematodes

The *C. elegans* strain used was Bristol N2. All experiments (including lifespan analyses) were performed exactly as previously described [17] except for streptomycin and 5-fluoro-2'-deoxyuridine, which were omitted. *E. coli* OP50 bacteria were heat-inactivated as previously described [17] for 45 min, and used as the only food source. Fat content and respiration rates were determined exactly as previously described [17].

received 03.11.2010

accepted 23.12.2010

Bibliography

DOI <http://dx.doi.org/10.1055/s-0030-1270524>
Published online: 2011
Horm Metab Res
© Georg Thieme Verlag KG
Stuttgart · New York
ISSN 0018-5043

Correspondence

M. Ristow
Department of Human Nutrition
Institute of Nutrition
University of Jena
07743 Jena
Germany
Tel.: +49/3641/949 630
Fax: +49/3641/949 632
mr@mrlistow.org

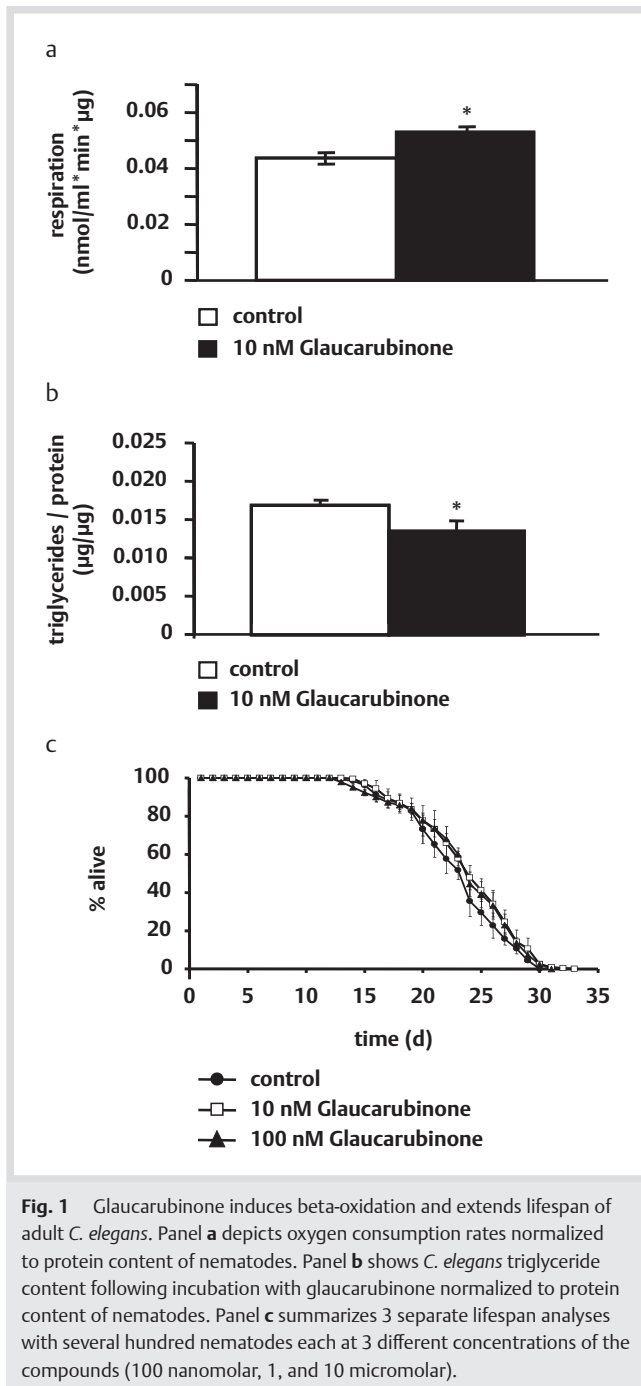


Fig. 1 Glaucaurubinone induces beta-oxidation and extends lifespan of adult *C. elegans*. Panel **a** depicts oxygen consumption rates normalized to protein content of nematodes. Panel **b** shows *C. elegans* triglyceride content following incubation with glaucaurubinone normalized to protein content of nematodes. Panel **c** summarizes 3 separate lifespan analyses with several hundred nematodes each at 3 different concentrations of the compounds (100 nanomolar, 1, and 10 micromolar).

Results

Glaucaurubinone increases respiration in *C. elegans*

We exposed *C. elegans* to a concentration of 10 nanomolar of glaucaurubinone for 2 days, and quantified oxygen consumption in a Clark-type electrode. In comparison to untreated nematodes, glaucaurubinone increases oxygen consumption in *C. elegans* (● Fig. 1a), consistent with an induction of oxidative metabolism.

Glaucaurubinone decreases fat content in *C. elegans*

Next, the question whether this induction of oxidative metabolism coincides with increased turnover of triglycerides, as previously observed for nematodes treated with 2-deoxyglucose was tested [17]. Indeed, we observed a reduction of fat content in

nematodes 72 h after initiation of glaucaurubinone treatment (● Fig. 1b), indicating that this compound reduces body fat in metazoans.

Glaucaurubinone extends *C. elegans* lifespan

As previously observed for 2-deoxyglucose, induction of respiration rates as well as reduction of body fat in adult nematodes suggests that glaucaurubinone may exert effects on *C. elegans* lifespan. Applying this compound at 2 different concentrations (10 and 100 nanomolar) to *C. elegans* using the above-mentioned methods extends life span significantly (● Fig. 1c). This effect appears not to be strictly dose-dependent, since no such correlation could be seen while both concentrations evaluated had a lifespan-extending effect. The maximum observable effect on mean life span was 1.9 days, which occurred at a concentration of 100 nanomolar. The maximum observable effect on maximum life span (80th percentile) was 2.7 days, which occurred at a concentration of 100 nanomolar.

Discussion

To potentially support the ongoing search for compounds that may promote human health especially at higher age, we show here that glaucaurubinone promotes longevity in a nematodal model organism, the roundworm *C. elegans*.

Glaucaurubinone is known to exhibit cell growth inhibiting [18–20] and antimalarial [21–23] activities. However the exact mechanism of action appears unresolved [24].

As shown in the present study, glaucaurubinone appears to induce mitochondrial activity and concurrently reduces body fat content. Another compound, 2-deoxyglucose, exerts very similar effects on *C. elegans*, as it has been mechanistically shown to extend nematodal lifespan by increasing production of reactive oxygen species (ROS) emanating from the mitochondria. These ROS act as a signal to ultimately increase endogenous antioxidant defense [25] and longevity [17], a signaling process that was previously named mitochondrial hormesis or mitohormesis [26]. Based on the current findings, it appears likely that glaucaurubinone acts by employing a similar mechanism.

Since the current study has been performed in the model organisms *C. elegans*, it is unclear whether our results can be extrapolated to mammals or even humans. Hence, further studies will have to show whether glaucaurubinone has any effect on mammalian health span and/or longevity. However, other compounds that have been identified by using a similar, metazoan-based approach have been shown to be effective in rodents [10–16]. Taken together, these findings indicate that glaucaurubinone induces mitochondrial metabolism, reduces fat content and extends lifespan in *C. elegans* suggesting that this compound may be worth evaluating in mammals and potentially humans in regard to prevention of aging and age-associated diseases.

Acknowledgements

The authors thank Beate Laube, Annett Müller, and Waltraud Scheiding for excellent technical assistance. This work was supported in part by a grant from the German Bundesministerium für Wirtschaft und Technologie (Federal Ministry of Economy and Technology) (BMW grant no. IW082104). Moreover, this work is part of the research program of the Jena Centre for Sys-

tems Biology of Ageing – JenAge funded by the German Ministry for Education and Research (Bundesministerium für Bildung und Forschung – BMBF; support code: 0315581).

Affiliations

- ¹ Department of Human Nutrition, Institute of Nutrition, University of Jena, Jena, Germany
- ² Analyticon Discovery GmbH, Potsdam, Germany
- ³ ProBioTec S. A. de C. V., San Salvador, C.G., El Salvador
- ⁴ Botanical Garden and Botanical Museum Berlin-Dahlem, Free University of Berlin, Berlin, Germany
- ⁵ Department of Clinical Nutrition, German Institute of Human Nutrition Potsdam-Rehbrücke, Nuthetal, Germany

References

- 1 Friedman DB, Johnson TE. A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* 1988; 118: 75–86
- 2 Kenyon C, Chang J, Gensch E, Rudner A, Tabtiang R. A *C. elegans* mutant that lives twice as long as wild type. *Nature* 1993; 366: 461–464
- 3 Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 2001; 292: 107–110
- 4 Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. *Nature* 1996; 384: 33
- 5 Holzenberger M, Dupont J, Ducos B, Leneuve P, Geloan A, Even PC, Cervera P, Le Bouc Y. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 2003; 421: 182–187
- 6 Blüher M, Kahn BB, Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 2003; 299: 572–574
- 7 Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* 1997; 277: 942–946
- 8 Weindruch R, Walford RL. The retardation of aging and disease by dietary restriction. Springfield, Illinois: Charles C Thomas Pub Ltd; 1988
- 9 Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, Weindruch R. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* 2009; 325: 201–204
- 10 Vellai T, Takacs-Vellai K, Zhang Y, Kovacs AL, Orosz L, Muller F. Genetics: influence of TOR kinase on lifespan in *C. elegans*. *Nature* 2003; 426: 620
- 11 Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon RA. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 2009; 460: 392–395
- 12 Kaeberlein M. Resveratrol and rapamycin: are they anti-aging drugs? *Bioessays* 2010; 32: 96–99
- 13 Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG, Zipkin RE, Chung P, Kisilewski A, Zhang LL, Scherer B, Sinclair DA. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* 2003; 425: 191–196
- 14 Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D. Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* 2004; 430: 686–689
- 15 Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006; 444: 337–342
- 16 Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, Swindell WR, Kamara D, Minor RK, Perez E, Jamieson HA, Zhang Y, Dunn SR, Sharma K, Pleshko N, Woollett LA, Csiszar A, Ikeno Y, Le Couteur D, Elliott PJ, Becker KG, Navas P, Ingram DK, Wolf NS, Ungvari Z, Sinclair DA, de Cabo R. Resveratrol Delays Age-Related Deterioration and Mimics Transcriptional Aspects of Dietary Restriction without Extending Life Span. *Cell Metab* 2008; 8: 157–168
- 17 Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab* 2007; 6: 280–293
- 18 Ghosh PC, Larrahondo JE, LeQuesne PW, Raffauf RF. Antitumor plants. IV. Constituents of *Simarouba versicolor*. *Lloydia* 1977; 40: 364–369
- 19 Ogura M, Cordell GA, Kinghorn AD, Farnsworth NR. Potential anticancer agents vi. Constituents of *Ailanthus excelsa* (Simaroubaceae). *Lloydia* 1977; 40: 579–584
- 20 Valeriote FA, Corbett TH, Grieco PA, Moher ED, Collins JL, Fleck TJ. Anticancer activity of glaucarubinone analogues. *Oncol Res* 1998; 10: 201–208
- 21 Trager W, Polonsky J. Antimalarial activity of quassinoids against chloroquine-resistant *Plasmodium falciparum* in vitro. *Am J Trop Med Hyg* 1981; 30: 531–537
- 22 O'Neill MJ, Bray DH, Boardman P, Wright CW, Phillipson JD, Warhurst DC, Gupta MP, Correya M, Solis P. Plants as sources of antimalarial drugs, Part 6: Activities of *Simarouba amara* fruits. *J Ethnopharmacol* 1988; 22: 183–190
- 23 Kirby GC, O'Neill MJ, Phillipson JD, Warhurst DC. In vitro studies on the mode of action of quassinoids with activity against chloroquine-resistant *Plasmodium falciparum*. *Biochem Pharmacol* 1989; 38: 4367–4374
- 24 Beutler JA, Kang MI, Robert F, Clement JA, Pelletier J, Colburn NH, McKee TC, Goncharova E, McMahon JB, Henrich CJ. Quassinoid inhibition of AP-1 function does not correlate with cytotoxicity or protein synthesis inhibition. *J Nat Prod* 2009; 72: 503–506
- 25 Ristow M, Zarse K, Oberbach A, Klötting N, Birringer M, Kiehnopf M, Stumvoll M, Kahn CR, Blüher M. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci USA* 2009; 106: 8665–8670
- 26 Ristow M, Zarse K. How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Exp Gerontol* 2010; 45: 410–418