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Secondary Plant Metabolites as Putative Modulators of Desaturases – Studies in Cultured Cells and Laboratory Mice

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Secondary Plant Metabolites as Putative Modulators of Desaturases – Studies in Cultured Cells and Laboratory Mice

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Summary

It has been hypothesized that the long-chain omega-3 fatty acids (FA) eicosapentaenoic and docosahexaenoic acid may contribute to diminish the risk of chronic diet-related diseases. The class of sphingolipids is mainly studied concerning its regulatory characteristics between cell death and survival. Desaturases insert double bonds in the carbon chain of FAs in the synthesis of both, omega-3 FAs and sphingolipids. The dietary modulation of these desaturases to increase the endogenous synthesis of FAs has been insufficiently studied until today.

Therefore, it was the aim of the present study to estimate the influence of different secondary plant metabolites on the modulation of desaturases and therefore the lipid metabolism. To this end, the impact of twelve flavonoids (apigenin [API], biochanin A, daidzein, equol, fisetin, formononetin, luteolin, genistein, orobol, pratensein, prunetin, and quercetin) and three stilbenes (resveratrol [RSV], lunularin, and dihydro-resveratrol) on the mRNA steady state levels of Δ4-, Δ5-, and Δ6-desaturases (DEGS1, FADS1, and FADS2, respectively) was evaluated in HepG2 hepatocytes, C21C12 monocytes, and 3T3-L1 adipocytes. Subsequently, two effective compounds (RSV and API) were studied in more detail in hepatocytes.

Resveratrol increased the expression of FADS1 and FADS2 *in vitro*. However, since this induction did neither increase concentrations of FADS protein, nor eicosapentaenoic, nor docosahexaenoic acid *in vitro* or in laboratory mice, RSV seems to inhibit desaturases post-transcriptionally. The application of RSV led to increased levels of saturated FAs *in vitro*, whereas it enhanced amounts of monounsaturated FAs *in vivo*. Both indicate a potentially stress-reducing influence of RSV by decreasing levels of polyunsaturated FAs.

The induction of DEGS1 via 20 µM API *in vitro* was potentially induced by a short-term stress signal after API-treatment. Increased expression of sphingosine kinase 1 indicates a potential synthesis of sphingosine-1-phosphate. Together with the receptor protein kinase EGFR (epidermal growth factor receptor), sphingosine-1-phosphate has been related to cell survival. Therefore, the strong induction of EGFR expression in the present study could induce subsequent EGFR-modulated signaling cascades, which favor cell growth and inhibit apoptosis. It was shown in this study that a high dose of 200 µM API decreased the expression of DEGS1 and sphingosine kinase 1 and led to cell death, which indicates a potential relation between sphingolipid synthesis and cell survival.

The experimental inhibition of the activity of receptor interactive protein kinase 1 and the simultaneous application of 20 µM API led to cell death and decreased the expression of genes encoding proteins involved in sphingolipid synthesis. Therefore, a relationship between receptor interactive protein kinase 1 activity and subsequent synthesis of survival-promoting sphingolipids is assumed.

In general, secondary plant metabolites seem to have regulatory impacts on the expression of desaturases, although dose and species-specific effects were observed. An induction of FADS1 and FADS2 and a subsequent potentially higher concentration of unsaturated omega-3 FAs are desirable from a nutritionist's point of view. Therefore, the effective substances of this study – daidzein, genistein, pratensein, and quercetin – need to be studied in more detail. Resveratrol led to an induction of FADS1 and FADS2 *in vitro*. However, it did not modulate hepatic levels of eicosapentaenoic or docosahexaenoic acid either *in vitro* or *in vivo*. Depending on the regulation of subsequent enzymes, an induction of DEGS1 may lead to the synthesis of both pro- and anti-apoptotic sphingolipids. In the present study, results indicate modulating characteristics of API on sphingolipid-synthesis *in vitro*.

Zusammenfassung

Die vorliegende Dissertation hatte das Ziel, den Einfluss verschiedener sekundärer Pflanzenstoffe auf die Modulation von Desaturasen und damit den Lipidstoffwechsel abzuschätzen. Dies lag darin begründet, dass die langkettigen Omega-3-Fettsäuren Eicosapentaen- und Docosahexaensäuren potenziell dazu beitragen können, das Risiko ernährungsbedingter Krankheiten zu senken. Für die Klasse der Sphingolipide werden darüber hinaus wichtige regulatorische Funktionen im Kontext von Zelltod und –überleben diskutiert. Desaturasen sind sowohl an der Synthese von Omega-3-Fettsäuren als auch von Sphingolipiden beteiligt, indem diese Doppelbindungen in die Kohlenstoffkette der Fettsäuren einfügen. Die diätetische Modulation dieser Desaturasen zur endogenen Fettsäuresynthese ist bislang unzureichend untersucht.

In einem initialen Screening wurde die Wirkung von zwölf Flavonoiden (Apigenin [API], Biochanin A, Daidzein, Equol, Fisetin, Formononetin, Luteolin, Genistein, Orobol, Pratensein, Prunetin, und Quercetin) und drei Stilbenen (Resveratrol [RSV], Lunularin, und Dihydro-Resveratrol) auf die mRNA steady state Level der Desaturasen 4, 5 und 6 (DEGS1, FADS1 und FADS2) in Hepatozyten, Myozyten und Adipozyten determiniert. Zwei effektive Substanzen (RSV und API) wurden nachfolgend detaillierter in Hepatozyten erforscht.

Es zeigte sich, dass RSV *in vitro* die Transkription von FADS1 und FADS2 induzierte und somit potenziell einen positiven Einfluss auf Omega-3-Fettsäure-Spiegel haben könnte. Da diese Induktion jedoch weder *in vitro* noch anschließend in Mäusen *in vivo* zu höheren Konzentrationen an FADS-Protein, Eicosapentaen- oder Docosahexaensäure führte, scheint RSV Desaturasen posttranskriptionell zu hemmen. *In vitro* resultierte die RSV-Applikation in erhöhten Konzentrationen gesättigter Fettsäuren, während die *in vivo* Gabe zu mehr einfach ungesättigten Fettsäuren führte. Beides spricht für potenziell stressmindernde Einflüsse von RSV durch die Senkung der Gehalte mehrfach ungesättigter Fettsäuren.

Während 20 µM API verschiedene Gene des Sphingolipid-Metabolismus induzierte, aber Zellüberleben nicht beeinflusste, hemmten 200 µM die Sphingolipid-Synthese und führten zu Zelltod. Die experimentelle Hemmung der Receptor interacting protein 1-Kinase oder der Katalase führten bei jeweils gleichzeitiger Gabe von 20 µM API zu Zelltod und verringerte außerdem die Exprimierung der Gene, welche für Proteine kodieren, die an der Synthese von Sphingolipiden beteiligt sind. Apigenin scheint dosisabhängige hormetische Effekte auf einige Gene der Sphingolipid-Synthese zu haben. Der potenzielle Anstieg an Sphingosin-1-Phosphat, welches mit Zellüberleben assoziiert wird, sowie die Induktion der Rezeptorproteinkinase EGFR (epidermal growth factor receptor) könnten möglicherweise Zelltod verhindern. Des Weiteren zeigte das Zellüberleben nach der Behandlung mit 20 µM API einen Zusammenhang zu der Receptor interacting protein 1-Kinase und der Katalase.

Generell scheinen sekundäre Pflanzenstoffe regulatorische Effekte auf die Exprimierung von Desaturasen zu haben, wobei sowohl Dosis-, als auch Zelltyp-Abhängigkeiten zu beobachten waren. Die Substanzen Daidzein, Genistein, Pratensein und Quercetin, welche in dieser Studie *in vitro* die Transkription von Desaturasen induzierten, sollten dahingehend noch weiter erforscht werden. Resveratrol führte zwar *in vitro* zu einer Induktion von FADS1 und FADS2, steigerte aber weder *in vitro* noch *in vivo* die Lebergehalte an Eicosapentaen- und Docosahexaensäure. Apigenin zeigte sich *in vitro* als möglicher Modulator der Sphingolipid-Synthese.

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