A PCR-BASED SCREEN TO IDENTIFY NATURAL COMPOUNDS WITH THE ABILITY TO INFLUENCE NRF2-MEDIATED TRANSCRIPTIONAL INDUCTION OF DETOXIFICATION/ANTIOXIDANT GENES

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ABSTRACT

It is widely accepted that oxidative injury and inflammation are intimately involved in the aging process and the development of age-related conditions. To date, most anti-aging strategies have focused solely on the delivery of exogenous antioxidants to combat the negative effects of aging. A promising new strategy is to identify nutrients and plant extracts that can directly target intrinsic cytoprotective mechanisms including: 1) upregulation of genes involved in the detoxification of xenobiotic and xenobiotic metabolites, 2) upregulation of genes involved in the synthesis and regulation of intrinsic antioxidants and antioxidant enzymes and 3) modulation of genes involved in the regulation of inflammation. Therefore, the purpose of this study was to evaluate natural compounds for the ability to modulate a representative panel of genes from key age-related pathways: the Nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE)/Phase II detoxification and inflammatory pathways.

Nrf2 is a transcription factor that regulates the basal and inducible expression of a large battery of genes encoding for cytoprotective factors including those that defend against electrophilic stressors and oxidative insults. We selected a panel of 8 genes representative of the Nrf2 and inflammatory pathways based on a review of the literature. Mice (C57Bl/6), aged 8 weeks were fed an AIN 93M diet without (n= 8) or with one of 12 compounds (n = 8/group) for 3 months. In a second, exploratory study, we compared the effects of a subset of compounds in young mice (8 weeks) and old mice (14 weeks) with and without supplementation for 16 months. The compounds included a variety of nutrients and plant extracts. Tissues were collected and gene expression of the 8-gene panel was analyzed by RT-qPCR. Finally, nutrients and plant extracts were ranked based on the number of Nrf2 genes upregulated, number of inflammation-related genes downregulated and the robustness of the changes in gene expression.

In summary, we have identified a robust panel of genes representative of the Nrf2 and inflammatory pathways that can be used as a rapid screening tool to evaluate the effects of specific nutrients on cellular detoxification, antioxidant status and inflammatory balance. This technique affords an opportunity to define the optimal blend of ingredients that can oppose gene expression changes in these key pathways that directly relate to human aging.

INTRODUCTION

- Aging is associated with the accumulation of cellular waste and damage over time and impairments in cytoprotective and repair mechanisms.
- Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that regulates the basal and inducible expression of a large battery of genes encoding for cytoprotective factors including those that defend against electrophilic stressors and oxidative insults.
- The Nrf2/antioxidant response element (ARE) antioxidant protection and Phase II Detoxification pathways are impaired with aging due to age-related changes in gene expression. A key example is the reduction in glutathione (GSH) levels in all tissues with age due primarily to declines in GCL and glutathione synthase (GS) expression (1).
- Aging is also associated with inflammatory dysregulation and changes in expression of inflammatory related genes.
- The purpose of this study was to evaluate natural compounds for their ability to modulate a representative panel of genes for key age-related pathways: the Nrf2/ARE cytoprotective and inflammatory pathways.

METHODS

STUDY 1. N = 8 MICE/GROUP:
Controls (C); 8 – 22 wks of age
Treated (T); 8 – 22 wks of age

STUDY 2. N = 5 MICE/GROUP:
Young Control (YC); 8 – 22 wks of age
Old Control (OC); 14 – 30 months of age
Old Treated (OT); 14 – 30 months of age

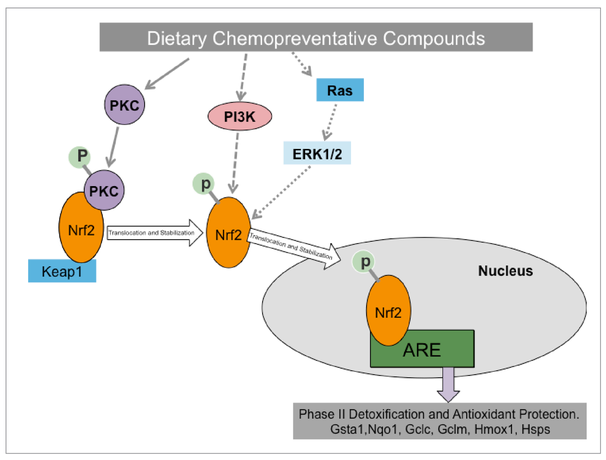
References

1. Lu Mol. Apsects. Med. 2009.
2. Barger et al. PLoSOne 2008.
3. Chen and Kong, FRBM:36; 2004
4. Suh et al. PNAS 2004.

INGREDIENTS TESTED

Compound 1. Low molecular weight amphiphilic antioxidant (LWAO)
Compound 2. Proprietary herbal extract 1 (PHE1)
Compound 3. Proprietary Citrus sinensis extract (CSE)
Compound 4. Proprietary fruit extract 1 (PFE1)
Compound 5. Investigational Compound 1 (IC1)
Compound 6. Cherry extract (CE)
Compound 7. Proprietary fruit extract 2 (PFE2)
Compound 8. Red wine extract (RWE)
Compound 9. Proprietary vegetable extract (PVE)
Compound 10. Curry extract (CUE)
Compound 11. Proprietary herbal extract 2 (PHE2)
Compound 12. Investigational Compound 2 (IC2)

Figure 1. Overview of the signaling cascade regulating Phase II Detoxification and Antioxidant Protection adapted from Chen and Kong (3).



GENES SCREENED

GCLC. Encodes for the heavy catalytic subunit of glutamate-cysteine ligase (GCL), also known as gamma-glutamylcysteine synthetase, the first rate limiting enzyme of glutathione synthesis.
GCLM. Encodes for the light regulatory subunit of GCL, also known as gamma-glutamylcysteine synthetase, the first rate limiting enzyme of glutathione synthesis.
GSR. Encodes glutathione reductase, a member of the class-I pyridine nucleotide-disulfide oxidoreductase family.
GSTA1. Encodes a glutathione S-tranferase belonging to the alpha class.
NQ01. A member of the NAD(P)H dehydrogenase (quinone) family and encodes a cytoplasmic 2-electron reductase.
UGT1A6. Encodes a UDP-glucuronosyltransferase, an enzyme of the glucuronidation pathway.
PTGS2 (COX2). Encodes for the inducible prostaglandin-endoperoxide synthase (PTGS2), also known as cyclooxygenase 2, the key enzyme in prostaglandin biosynthesis.
Nos2 (iNOS). Encodes for inducible nitric oxide synthase which is expressed in liver.

RESULTS

TABLE 1
Changes in gene expression in liver following short-term feeding of compounds. C vs. T

LIVER

	Gclc	Gclm	Gsr	Gsta1	NQO1	UGT1A6A	Ptgs2	NOS2
Ctrl	1.01	1.01	1.01	1.06	1.04	1.01	1.39	1.11
LWAO	1.18	1.31	1.33	3.41	1.09	1.24	1.02	1.03
PHE1	-1.11	1.28	-1.03	3.23	1.17	1.10	-1.22	-1.03
CSE	1.09	1.60	1.47	4.86	1.25	1.10	1.05	1.01
PFE1	-1.04	1.12	1.14	2.94	1.19	1.03	1.50	1.37
IC1	-1.17	1.26	1.03	2.38	-1.21	1.01	-1.21	-1.01
CE	-1.13	1.00	-1.12	2.53	-1.02	1.05	1.69	2.98
PFE2	-1.02	1.08	1.03	3.54	1.22	1.19	1.11	1.14
RWE	-1.00	1.23	1.08	3.26	1.47	1.09	1.79	1.45
PVE	-1.03	1.13	1.01	2.84	1.77	1.15	1.68	1.46
CUE	1.08	1.30	1.14	2.45	-1.05	1.19	-1.17	1.47
PHE2	-1.03	1.19	-1.13	1.93	1.46	1.04	2.17	2.00
IC2	-1.22	-1.18	-1.24	1.29	-1.32	-1.04	1.92	1.14

Statistically significantly upregulated compared to control; p < 0.05

Statistically significantly downregulated compared to control; p < 0.05

TABLE 2
Changes in gene expression in gastrocnemius following long-term feeding of compounds in YC vs. OC and OC vs. OT

GASTROCNEMIUS MUSCLE

	Gclc	Gclm	Gsr	NQO1
YC	1.01	1.01	1.02	1.02
OC	-1.10	-1.02	-1.36	-1.06
LWAO	1.58	1.00	2.10	-1.04
PFE1	-1.11	1.56	-1.16	-2.86
IC1	1.41	1.01	1.41	-1.21
CUE	1.40	-1.33	1.40	-1.95
IC2	1.39	1.59	1.43	-2.18

Statistically significantly upregulated compared to old control; p < 0.05

Statistically significantly downregulated compared to old control; p < 0.05

TABLE 3
Comparison of changes in gene expression in a subset of ingredients studied in both liver and gastrocnemius muscle

LIVER

	Gclc	Gclm	Gsr	NQO1
Ctrl	1.01	1.01	1.01	1.04
LWAO	1.18	1.31	1.33	1.09
PFE1	-1.04	1.12	1.14	1.19
IC1	-1.17	1.26	1.03	-1.21
CUE	1.08	1.30	1.14	-1.05

GASTROCNEMIUS MUSCLE

	Gclc	Gclm	Gsr	NQO1
YC	1.01	1.01	1.02	1.02
OC	-1.10	-1.02	-1.36	-1.06
LWAO	1.58	1.00	2.10	-1.04
PFE1	-1.11	1.56	-1.16	-2.86
IC1	1.41	1.01	1.41	-1.21
CUE	1.40	-1.33	1.40	-1.95

Statistically significantly upregulated; p < 0.05

Statistically significantly downregulated; p < 0.05

SUMMARY & CONCLUSION

STUDY 1. SHORT TERM-TERM FEEDING IN YOUNG

- The greatest effects on gene expression were seen with LWAO, CSE, PVE and PFE2.
- Some ingredients, such as PHE2 appeared to have no benefit and actually increased expression of the pro-inflammatory gene Ptgs2, suggesting a potential negative effect of this ingredient.

STUDY 2. LONG-TERM FEEDING IN YOUNG AND OLD

- Consistent with Study 1 results and the literature (3), the greatest effects on gene expression were seen with LWAO.
  - LWAO effectively opposed age-related declines in Gclc and Gsr expression.
- IC1 effectively opposed age-related decreases in both catalytic subunits responsible for GSH synthesis, Gclm and Gclc.

GENE PANEL

- Differential results were observed in Gclc and Gclm expression in YT, OT and in the different tissues as has reported previously (1).
- Gsta1 appears to be modulated by a number of compounds.
- It is important to account for age, length of feeding in study design and to evaluate multiple tissues AND multiple genes when screening ingredients.

In summary, we have identified a robust panel of genes representative of the Nrf2 and inflammatory pathways that can be used as a rapid screening tool to evaluate the effects of specific nutrients on age-related changes in cellular detoxification, antioxidant status and inflammatory balance. This technique affords an opportunity to rapidly identify compounds of interest and then to define the optimal blend of ingredients that may oppose genetic changes in these key pathways that are directly related to human aging.

Ongoing studies are utilizing genome wide gene expression profiling in brain, muscle, liver and other tissues using microarray technology to investigate potential effects of blends of natural ingredients in opposing the effects of aging.

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