Changing rules re the use of botanicals in NIH-funded research

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The composition of botanicals

Botanicals can be the specific parts of a plant, a simple extract of the plant part, or a purified component

- e.g., green tea (powdered dry leaves of the *Camellia sinensis*)
- Green tea polyphenols (a dried, concentrated water extract of green tea enriched in tea catechins)
- Epigallocatechin-3-gallate (purified green tea catechin with alleged highest antioxidant activity)
Puerarin (PN)
Study reproducibility

• Systematic science predicts that a single compound or even a complex matrix with a defined and constant composition will yield the same biological response assuming that all other experimental conditions are carefully controlled.

• If the botanical preparation is defined and has a constant composition, it will behave just like a pharmaceutical agent.
Regulations re composition of botanicals

• The 1994 DSHEA act did not legislate a standard for the composition of botanicals
• The ODS initiated a program in 2002 to define validated methods for the analysis of the bioactive components in the most popular botanicals/dietary supplements (interagency agreement with the FDA which led to a contract to AOAC International)
• A second contract was established with NIST to prepare standardized botanicals/dietary supplements in specific matrices to be reference materials for analyses.
<table>
<thead>
<tr>
<th>AOAC Methods</th>
<th>On the PTFDS List</th>
<th>Constituents</th>
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</thead>
<tbody>
<tr>
<td><strong>Hawthorn (Crataegus spp.)</strong></td>
<td></td>
<td>Chlorogenic acid, epicatechin, hyperoside, isoquercitrin</td>
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<tr>
<td><strong>Biotin</strong></td>
<td></td>
<td>Parthenolide(s)</td>
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<td><strong>Feverfew</strong></td>
<td></td>
<td>Pyrrolizidine alkaloids (PA’s)</td>
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<td><strong>Comfrey</strong></td>
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<td><strong>Colostrum</strong></td>
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<tr>
<td><strong>Creatine</strong></td>
<td></td>
<td>Valerenic acid(s)</td>
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<tr>
<td><strong>Podophyllotoxins</strong></td>
<td></td>
<td>Baicalin</td>
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<tr>
<td><strong>Valerian</strong></td>
<td></td>
<td>Hydrastine, berberine, palmatine</td>
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<td><strong>Baical skullcap</strong></td>
<td></td>
<td>aucubin, agnuside, casticin, isovitexin, orientin</td>
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<tr>
<td><strong>Cardiac glycosides</strong></td>
<td></td>
<td>Polyphenols</td>
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<td><strong>Goldenseal</strong></td>
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<td><strong>Chaste tree</strong></td>
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<td><strong>Choline</strong></td>
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<td><strong>Pine bark extract</strong></td>
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AOAC Methods

Prioritized Methods (IRS)

- Red clover
- L-Carnitine
- B Vitamins
- Black cohosh
- ω-3 Fatty acids
- Soy isoflavones
- Green tea catechins
- Lutein
- Turmeric
- Ginger
- Milk thistle
- African plum
- Flax seed

Constituents

- Isoflavones
- Triterpene glycosides
- EGc, EGCG
- Curcumin
- Gingerol, shoagol
- Silymarin
- Fatty acids, phytosterols
- Secoisolariciresinol-diglucoside
AOAC Methods

In Progress (ERP)

- Ephedra*
- Aristolochic acids
- SAMe
- ß-Carotene*
- Chondroitin sulfate
- Glucosamine*
- St. John’s wort
- Ginkgo flavonols*
- Ginkgoterpenes
- Saw palmetto phytosterols
- Saw palmetto fatty acids
- Bitter Orange

Constituents

- Ephedrine, pseudoephedrine, 4 other alkaloids
- Aristolochic acids A and B
- Rutin, quercitrin, quercetin, isoquercitrin, hyperoside, pseudohypericin, hypericin, hyperforin
- Quercetin, kaempferol, isorhamnetin
- Bilobalide, Ginkgolides A, B, C, J
- Campesterol, stigmasterol, Beta-sitosterol
- Free fatty acids
- p-synephrine, tyramine, N-methyltyramine, octopamine, hordenine
Botanicals versus therapeutics

• Although we might think of classical therapeutics as pure compounds, many are not.
  – Many agents used to treat cancer are derived from plants (vinblastine, vincristine, taxol, etc.)
  – Premarin™ is an extract of horse urine and contains 8-10 estrogen-like compounds as well as many phenolic acids - its composition is regulated with specific ranges of its components defined

• Botanicals/supplements are rarely pure compounds (an exception is vitamin c)
  – Not always clear that the presumed bioactive is present
The Echinacea saga

- NCCAM funded a study of Echinacea and clinical immunity in children - this was published in JAMA - the study conclusion was that “Echinacea had no significant effect”
- Criticism of this study has included discussion about whether the correct part of the *Echinacea purpurea* was used, to the late intervention with this supplement
- Interestingly, there was a significant reduction in the number of second and third event colds in children treated with *E. purpurea* compared to controls. This was not reported by the media.
Reaction to the Echinacea saga

• NCCAM has created a set of new regulations that govern its funding of experiments in which botanicals or dietary supplements are used.

• It is therefore crucial to properly characterize the botanical you’re going to use in a study.

• You may wish to use a pure component derived from a botanical - NCCAM has rules for that, too.
NCCAM rules for botanicals

- See NOT-AT-05-004
- Evidence that a reproducible product is available
- Reserve test and control diets for later analysis
  - Provide a plan for sampling scheme, storing and analyzing samples, establishing variances and making results available to NCCAM
- The dose to be used should be justified
How NCCAM views the botanical

• The complex natural product (the botanical) is the purpose of studies

• Isolated constituents can be used if the intent is to
  – characterize or standardize the whole botanical
  – compare their activity to the whole botanical
  – determine the mechanism(s) of action
  – improve the preparation of the whole product

• NCCAM will not support development of isolated constituents as drugs
  – However, if a pure compound is already available as a dietary supplement, it qualifies for support (e.g., vit C)
In the text of the application

Descriptors

– **Name of the product (species, strain, as applicable);** *Vitis vinifera*

– **Parts to be used (e.g., root, stem, leaf) as applicable;** seed of the grape; purified to 90% purity (as polyphenols)

– **Description of placebo or vehicle control group;** the grape seed extract was added to AIN-76A diet. Control diets had no added grape seed extract

– **Doses or concentrations to be used.** Previous toxicity studies in Dr. Kim’s lab had suggested that 5% GSE in the diet was non-toxic. The goal of the proposed study was to examine GSE’s effects down to a dose of 0.1% (by wt.) in diet.
In the Background and Significance section, provide the following:

- Justification/rationale for studying the chosen product;
- Justification for the chosen form of the product (extract, powder, etc.);
- Justification for the proposed doses/concentrations;
- Description of the pharmacokinetics of the product (if known);
- Source of the product (and if not using a commercial source, an explanation of why a product generally available to the public is not being used).
Just-in-time issues

• If the application has a priority score that gives it a likelihood of being funded, NCCAM will notify the PI to provide product quality information
• “Unsatisfactory” information may prevent funding
  – Applicant may have to re-apply and submit additional information for the next review cycle
Approaching this in a recent R21 application

The potential for plant polyphenols to selectively mimic hormone replacement therapy in maintaining cognition

- *What has been the standard hormone replacement therapy (Premarin™) for 40-50 years, has been proved to be ineffective in preventing heart disease*

- *Experiments with diets supplemented with other proanthocyanidins in fruits and berries have shown improvements in cognitive function induced by ovariectomy*
Use of grape seed extract

Grape seed extract (GSE) is a widely available dietary supplement marketed for anti-oxidant health benefits.

The GSE material forming Gravinol™ was supplied by Kikkoman Corp. It was 90% by weight of oligomeric PACNs and 7% by weight of other polyphenols including catechin monomers.
GSE and health

• Documented history of health benefits of GSE and related PACNs in cognitive dysfunction
  – Peng (2005) found that 0.5% GSE enhanced cognitive function in OVEX spontaneously hypertensive rats
  – Deshane et al. (2004) showed effects on brain protein expression from 5% GSE in the diet

• Role for PACNs in other models of human chronic diseases:
  – Prevention of gastric erosions due to stress (Iwasaki, 2004)
  – Lens cataract disease in ICR/f rats (Yamasoki, 2002) and diabetic rats (Osakabe, 2004; El-Alfy, 2005)
  – Prevention of colonic cancer (Nomoto, 2004; Gosse, 2005)
Dose selection of GSE

- Rationale for systematic analysis of dose response of GSE in animal studies, and dose selection (0, 0.05, 0.1, 0.25, 1.0 and 2.5%)
  - Previous studies had shown that when 5% GSE was given to young adult rats, brain proteins were systematically changed (Deshane, 2004)
  - 0.5% GSE improves cognitive function in OVEX SHR (Peng, 2005)
  - 0.2% GSE slows onset of lens cataract disease in ICR/f rats (Yamasokki, 2002)
  - 0.1% GSE is equivalent to human dosage from supplements on a per kg body weight basis
Safety and Toxicity

- Safety and toxicity of GSE PACN
  - Kim (2004) found that there were no systematic changes in organ weights up to 10% GSE in the diet in rats
  - Yamasoki (2002) observed a no-observed-adverse-effect level (NOAEL) of 2% in the diet
  - Bentivegna (2002) proposed a NOAEL of 2.5%
Known pharmacokinetics of the product to be used

• Current knowledge of the pharmacokinetics of GSE PACN
  – The flavonols present are quickly absorbed, methylated, sulfated and glucuronidated
  – Half lives are ~ 4 h, ring-opened metabolites have longer half lives (Takizawa, 2003)
  – The oligomeric PACNs are poorly absorbed; small amount of catechin dimers from chocolate (Holt, 2002), and higher oligomers (Shoji, 2006) from apple PACNs; their tetramethyl ethers (Garcia-Ramirez, 2006) found in blood following administration of synthetic PACNs
Choice of GSE and the model

- Rationale for the study of actions of GSE polyphenols in the spontaneously hypertensive rat (SHR)
  - The SHR model is well-established
  - Peng (2005) found that 0.5% GSE improved cognitive function in OVEX SHR rats
  - Others have reported that PACNs improve cognitive function
What is in the diet in the experiment

- Analysis, validation and monitoring of GSE and diet composition over the course of the study
  - This will be covered by Dr. Prasain in his section
Making and storing the diet
Diet mixing and storage

- Both liquids and solids can be blended into the diet - must be well mixed - batch lots of ~9 kg
- Diets stored both in the dark and at -20°C
- Normally diet used over a 4-6 week period
- New NCCAM rules require tests of the stability of the added compounds in the diet both at room temperature and after cold storage
- Diet must be retained for post study analysis
Acknowledgements

• Joseph Betz - Office of Dietary supplements at NIH
• Clinton Grubbs, PhD - UAB
• Helen Kim, PhD - UAB
Bibliography