SC-2

Use of *in vitro* assays for evaluating physiological functionality of foods: General consideration

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Abstracts

A new health paradigm may be evolving that would place emphasis on the positive aspects of diet, identifying components that are physiologically active and that contribute to the prevention of disease onset. It has been increasingly difficult to evaluate the impact of new bioactive materials and food products on the well being of society. Thus, testing systems for both health function and toxicity have become very elaborate, complex, and interrelated, making their interpretation difficult and open to controversy. To select the proper starting materials, to screen the appropriate health functionality and to determine the efficacy of product, a reliable, reproducible, sensitive and predictive assay is required. Particularly, *in vitro* assay is the first stage in preclinical test on physiologically active materials and have many advantages in terms of time, cost and convenience. However, several factors as well as some limitations should be considered in this assay system. This presentation, therefore, will address the use of *in vitro* assays for evaluating physiological functionality of foods coupled with general consideration.

Introduction

A growing number of products are being promoted as having health benefits. A recent survey of the top 100 food companies in the United States identified functional foods/nutraceuticals as the single most important consumer trend impacting new food of food ingredient. In addition, physiologically active chemicals should be examined as thoroughly as needed to initiate health claims required by the law started in South Korea. In attempt to select the proper starting materials, to screen the appropriate health functionality and to determine the efficacy of product, a reliable, reproducible, sensitive and predictive assay is required. Particularly, *in vitro* assay is the first stage in preclinical test on physiologically active materials and have many advantages in terms of time, cost and convenience. However, several factors as well as some limitations should be considered in this assay system.

Use of *in vitro* assays

An assay is a test, and the test must be reliable, reproducible, sensitive, meaningful and, most importantly, predictive. It is absolutely necessary to put the assays to be discussed in the context of their usage before one can evaluate them or understand their benefits and weaknesses. The use of complex *in vivo* assays for the initial evaluation of randomly selected crude extracts would certainly be an expensive project virtually doomed to fail unless one was lucky enough to get a hit of extremely low probability in the first few hundred experiments. In discovery of bioactive materials and functional foods, definitions and major roles of assays are given in Table 1.

Not all discovery programmes contain separate prescreens and screens, since if assays have enough capacity and are selective enough to reduce leads to a manageable number for the secondary testing to follow, prescreens are not needed. The monitor may be the same as the prescreens or the screen or may be a surrogate which can be done more

Table 1. Roles of bioassays in discovery of bioactive materials and functional foods

Step used	Definition	Consideration & description
Prescreen	An assay applied to large numbers of initial samples to determine whether or not they have any cancer preventive activity of the desired type.	- must have high capacity - must have low cost - must give rapid answer - need not be quantitative - for discarding inert materials and for providing an enriched feedstock for the screen
Screen	An assay which is used to select materials for detailed individual study (secondary testng).	- for selection to a manageable number for secondary testing
Monitor	An assay used to guide fractionation of a crude material towards isolation of the pure bioactive compounds.	- must be fast, cheap and high capacity - must be readily available to the chemist
Secondary testing (detailed evaluation)	Careful, detailed testing of lead compounds in multiple models and test conditions to select candidates for development toward clinical test.	- characteristically, low capacity, expensive and slow assay
Clinical trials	Phase I (toxicology study, preclinical trial) Phase II (limited trials to evaluate activity against specific cancers) Phase III (larger trials against a greater variety of cancers to compare the activity of the new agent with standard therapy)	- chosen for phase III, if agents show activity in phase II.

readily or can done using simple facilities in a chemistry laboratory. One of the most difficult logistical problems in assay or bioactivity-directed isolation from crude extracts is having test capacity available for fractions at the time when it is needed and giving rapid turnaround of data back to the chemist. The decision to combine chromatographic fractions based on chemical similarity cannot be made until the fractions are available so the number of the test samples for bioassay cannot be predicted in advance. In an ideal system, a certain amount of capacity for testing chromatographic fractions is reserved in the prescreening or screening laboratory and last-minute adjustments are made by addition or subtraction of crude extracts, This can badly muddle computerised test schedules, however, and alternatives are to have a separate biology laboratory to do only fractionation monitoring, or to use robust (often surrogate) assays which the chemical group can run themselves.

Generally, assays can best be divided into two groups: *in vitro* and *in vivo* assays. *In vitro* assays can be subdivided into two groups: cellular assays and molecular assays. Cellular assays use intact cells while molecular assays look for activity using isolated systems such as enzymes, receptors, DNA, etc. These assays have different characteristics in terms of target, assay capacity needed, number of candidates (leads) found, false positive and false negative. In particular, it is easy to obtain both false positive and false negative results in evaluating the physiological functionalities of health foods and materials due to their diverse physico-chemical characteristics.

When several samples and their constituents were tested for their cytotoxicity potentials in the routine culture condition, no reproducible and accurate results were obtained. For instance, volatile compounds such as diallyl sulfide ($(CH_2=CHCH_2)_2S$), diallyl disulfide ($(CH_2=CHCH_2)_2S_2$), diallyl trisulfide ($(CH_2=CHCH_2)_2S_3$) and garlic oil did not manifest the protective effects on B[a]P-induced toxicity in the routine culture condition. However, when hydroxypropyl-cyclodextrin was used to include volatile chemicals in the controlled culture condition, these volatile compounds and oil significantly increased the cell viability. By using 0.5% carboxymethyl cellulose, the sensitivity and reproducibility of cytotoxicity assay on insoluble samples were increased. These results suggest that special consideration on the assay conditions was needed to evaluate the physiological functionalities of health foods and materials with diverse physico-chemical characteristics *in vitro*.

Conclusion

A great variety of *in vitro* methods have been described in the literature, most of which yield poorly reproducible results, or are difficult and costly set up. The use of *in vitro* assay provides a well-suited and simple method that can both accomodate the need for a high throughput of new bioactive compounds and yield results which can already explore

the mechanism of action of the active compound. However, special consideration on the assay conditions will be needed to evaluate the physiological functionalities of health foods and materials with diverse physico-chemical characteristics *in vitro*. The major challenges in near future will include development of new *in vitro* assay and/or HTS system. Additionally, the development of *in vivo* models with the same level of molecular sophistication as the new *in vitro* models will also be required or evaluating physiological functionality of foods.

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