Assessing bioactivity

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In this month's issue of Pharmacognosy Research, I would like to concentrate on the methodologies that are employed to assess the nature of the bioactivities of plant extracts. *In vivo* and / or *in vitro* test models, together with the structural determination of the chief components, are of course the gold standard in determining the active constituents. Despite the development of very rapid and sophisticated analytical techniques, for example, Liquid chromatography (LC) and Gas chromatography (GC) hyphenated with Mass Spectrometry (MS) and elaborate one and two-dimensional Nuclear Magnetic Resonance (NMR) techniques, the bioassay remains indispensable. In general, bioassays examine the response of a whole animal, an isolated tissue / cell line or an organism, to a given extract (or drug, chemical or pollutant) in order to evaluate the bioactivity or toxicity or both. Paradoxically the difference between a 'cure' and a 'poison' is quantity. Scientists must be able to determine the activity (biological mechanisms) and the identity of the plant-borne substances in order to extrapolate safe levels, dosage forms, and delivery modes, for human use.

In this month's issue the brine shrimp lethality assay features in two of the articles. This is robust, simple, and inexpensive, and it is invaluable as a general frontline screen. It was introduced in 1982, by Meyer and co-workers[1] and has been successively employed for bioassay-guide fractionation of active constituents from many sources. The assay at its simplest involves taking brine shrimp eggs (*Artemia salina*), which are available commercially, and inducing the eggs to hatch into larvae; the hatching chamber is designed so as to automatically de-shell the hatchlings. A proportion of these naked larvae are then exposed to the diluted plant extract versus a control group. After an appropriate incubation period both groups are compared, usually by microscopy, to ascertain the LC_{50} (the lethal concentration of test material to half of the test organisms). This information can then be extrapolated to determine which fractions are bioactive, for example: crude extracts with LC_{50} values of less than, say 250 µg/ml may be deemed 'active' and retained for further, more refined tests.

The carrageenan-induced paw swelling assay (rat hind foot pad edema) is an old and reliable method for assessing inflammatory responses to antigenic substances[2] and also features in this month's issue. Carageenan is injected subcutaneously usually into the back paw causing an acute, non-immune reproducible inflammation, quantified by increase in paw size (maximal after ca. 5 hours). The inflammation is caused by the action of inflammatory agents in the rat: bradykinin, histamine, tachykinins, complement and reactive oxygen, and nitrogen species. The target compounds or extracts are then tested for their ability to reduce the swelling versus a control set, again simple, but effective.

Rat and mouse bioassays are still going strong, although they are a source of contention. These rodent assays are valuable for assessing the LD_{50} (median lethal dose of a substance, which will kill 50% of a given test population, expressed in toxin / Kg body weight; the lower the LD_{50} value the more toxic the substance) of test compounds; for determining the histological changes in key organs and tissues, in response to selected agents; and of course for performing genetic (so called 'gene knock out') studies, as well as for assessing the acute and chronic responses to xenobiotics, including toxins. However, there is a controversy surrounding the use of rodent bioassays, especially with regard to their use as predictors of human response, with the contention that they are often not reliable for short-term, long-term or life time studies.[3,4]

To state the obvious, there are too many fundamental differences between rodents and humans in terms of gene regulation, metabolic pathways, and immune responses that invalidate some extrapolations; however, these assays can be of value when used in conjunction with *other* testing models. In addition, there is an ethical concern among many

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people; rats and mice used in toxin / chemical screening are often subjected to very high doses of toxic compounds via intubations, forced inhalation or skin absorption, which undoubtedly causes suffering to the creatures, and consequently there is an impetus to find alternatives, mainly *in vitro* tests, polymerase chain reaction (PCR) technology, and predicative software.

Other common assays used for the assessment of bioactivity include antimicrobial assays, which determine if a selected compound(s) can kill or impede the growth of pre-selected microorganisms, including bacteria, viruses, and fungi. May I point out a very interesting article in this month's issue by Cock and Kalt, which investigates the potential of a modified MS2 bacteriophage reduction assay, which can rapidly (24 hours) and reproducibly determine antiviral activity in plant extracts.

Of course one of the key motivators today is the hunt for potential anti-cancer agents derived from plants, which is a very wide and interesting field, and a topic that we will return to in the near future.

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