Animal cells bioassays and bioproducts

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Introduction

Biological assays are methods for the estimation of nature, constitution, or potency of a material by means of the reaction that follows its application to living matter. Bioassay is defined as estimation or determination of concentration or potency of physical, chemical or biological agents by means of measuring and comparing the magnitude of the response of the test with that of standard over a suitable biological system under standard set of conditions [1,2]. An assay is a form of biological experiment; but the interest lies in comparing the potencies of treatments on an agreed scale, instead of in comparing the magnitude of effects of different treatments. Biological assays or biological standardizations or simply bioassays are methods used for estimation of the potency of substances by observing their pharmacological effects on living animals (in vivo) or isolated tissues (in vitro) and comparing the effect of these substances of unknown potency to the effect of a standard.

Bioassays are based upon the use of biological responses as detection system for biologically active substances. In the simplest form it is used to assay the presence (and concentration) of a particular substance by comparison with a known amount of the same substance. Both are procedures by which the potency or the nature of a substance is estimated by studying its effects on living matter. Bioassay is a procedure for the determination of the concentration of a particular constitution of a mixture [5-8].

Structure of biological assays

The typical bioassay involves a stimulus applied to a subject. Application of stimulus is followed by a change in some measurable characteristic of the subject, the magnitude of the change being dependent upon the dose. The intensity of the stimulus is varied by

using the various Doses by the analyst.

Principle of Bioassay

Active principle to be assayed should show the same measured response in all animal species [9]. Bioassay involves the comparison of the main pharmacological response of the unknown preparation with that of the standard [10-14]. The method selected should be reliable, sensitive, and reproducible and should minimize errors due to biological variation and methodology. The degree of pharmacological response produced should be reproducible under identical conditions. The reference standard and test sample should have same pharmacological effect and mode of action, so that their DRC curve run parallel and their potency ratio can be calculated [15-18]. Activity assayed should be the activity of interest; Individual variations must be minimized/accounted for [19]. Bioassay might measure a diff aspect of the same substance compared to chemical assay.

Types of Bioassays

There are three main types of bioassays (other than qualitative assays) [27]

1. *Direct Assays*
2. *Indirect Assays based upon quantitative responses*
3. *Indirect Assays based upon quantal responses („all or none")*

Direct Assay

Doses of the standard and test preparations are sufficient to produce a specified response, and can be directly measured.

Indirect Assay

In indirect bio-assays the relationship between the dose and response of each preparation is first ascertained. Then the dose corresponding to a given response is obtained from the relation for each preparation separately [29].

Quantal Assay

This response is in the form of „all or none" means no response or maximum response. These can be biossayed by end point method. Predetermined response is measured which is produced by threshold effect. Quantal Responses are population response based on an all-or-nothing (0 or 1 – presence or absence) response such as death [30-34].

Concentration of Unknown = Dose of the Standard Dose of the Test×Concentration of Standard

Graded Assay

It is proportional to the dose and response may lie between no response and maximum response [28]. Graded Responses can be any type of measured responses in isolated tissues in particular, but also in whole animals. Such responses are infinitely graded and there are a large number of them. Examples include contractions of muscle, blood pressure, blood sugar concentrations, etc. [35]

Matching Method

In this type of assay the test substance and the standard are applied and the responses obtained are matched by a trial and error process until they produce equal effects [36-38]. This may also limit to analytical dilution assay, as the assay involves the determination of the factor by which the test substance is diluted or concentrated in order to produce response that is equal to that of known amount of the standard drug [39-40]. Its advantage is that it does not depend on the assumption of a dose-response relationship. The main disadvantages are that it is purely subjective, and experimental errors cannot be determined from the assay. It gives no indication or the parallelism of the dose-response curves of the standard drug and test substance, and hence the qualitative differences, as the effects are matched at only one dose level. [41-46]

Advantages:

Quick and easy; useful when one is has many samples to test and a semiqualitative

answer is sufficient. Disadvantages: Inherently lacks precision, no accuracy, no D-R data – particularly no data regarding slope. The data is not easily statistically analyzed and probably should not be so analyzed [47].

Bracketing Method

Bracketing bioassay is performed by selecting two standard doses, which will give a close bracket on either side of the response produced by the unknown. The working dose of standard is first determined in the sensitive part of dose-response curve, that is, a dose that will approximately produce 50% of the maximal concentration. The dose of the standard drug is kept constant throughout the experiment, in order to have some idea about the change in the sensitivity of tissue with time. [48-53] The standard drug is added at fixed intervals but alternating with the test so that each response produced by a dose of test substance is bracketed by responses produced by the dose of standard. The response of test substance is bracketed between two responses of the standard. Close bracketing gives more accurate results. [54-56]

Interpolation Method

This is a simplest form of graded response assay and involves no statistical data and many calculations. In this assay the dose response curve is fist obtained from different doses of standard ach solution. The concentration of unknown is then read from the standard graph. [57-62] Interpolation method of bioassay is less time consuming and yet reliable compare to matching type of bioassay. One of the main advantages of this essay is that the sensitivity of the tissue is first determined by prior plotting of a dose response curve with a known agonist as in the case with acetylcholine. If the linearity of curve is good, one can do very accurate estimate of the test substance unknown sample.

Characteristics

A good bioassay should has the following characteristics

Sensitivity*- ability to detect smallest concentration*

Specificity*-the response which is being measured should be specific*

Reproducibility*-same observations by using different instruments and operators,over longer period of time*

Stability*-sensitivity of preparation should be constant and stable*

Availability*-the particular tissue or cell should be easily available*

Advantages

There are a number of advantages to including Maximum Tolerated Dose(MTD) in long-term animals bioassays

Interspecies comparison

When bioassays are conducted in more than one animal specie,use of MTD provides a consistent basis for interspecies comparisons

Sensitivity

The greater sensitivity of MTD are more likely to give positive or negative results than can be starting point for structural activity correlation analysis.

Preparations

Several preparations can be obtained from single animal cell

Cheap

Animal cells bioassays is a very cheap and less time consuming method than other methods

Disadvantages

Where there are advantages of animal cells bioassays ,there are some disadvantages of bioassays too

Non-specificity

Bioassay is non-specific and provides no information about biochemical and physiological mechanism during tumor production

Toxicity

It induces toxicity that leads to change in food consumptions,cytotoxicity in specific organs and hormonal imbalance.

Cross contamination

There is high possibility of cross-contamination of different types of cells while working with bioassays

Identification

Identification of cell type is often difficult in most of the cases,the markers proteins are not expressed under in-vitro conditions

Applications

The applications of animal cells bioassays are as follows

Detection and isolation of proteins

Bioassays is used for the detection and isolation of proteins such as,somatotrophin,insulin-like growth factors(somatomedins) ,insulin and transferrin etc

Detection of muscle growth factors

Radioimmunoassays cannot be used effectively for the detection and characterization of unknown and poorly characterized muscle growth factor.so for such detection bioassays are capable and reliable method that detects those factors that influencing the muscle growth.

Potency of agents

Animal cells bioassay is used to estimate the potency of agents and their effects.

Cytotoxicity studies

Bioassay is used to check the in-vitro toxicity of compounds or drugs in animals cells.

Therapeutic products

Bioassays are used to establish the acticity of therapeutic products.

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