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Bioassay- An Important Pharmacological Tool

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ABSTRACT

Bioassay is the determination of the relative strength of a substance by comparing its effect on a test organism with that of a standard preparation. It is typically conducted to measure the potency of a new compound, examine effects of the substance on a living organism, essential in the development of new drugs and in monitoring environmental pollutants. Bioassays are broadly of two types: qualitative and quantitative. Qualitative bioassays are used for assessing the physical effects of a substance that may not be quantified, such as abnormal development or deformity. Quantitative bioassays involve estimation of the concentration or potency of a substance by measurement of the biological response that it produces. Bioassay can again be categorized into quantal and graded response. The main aim of bioassay is to provide information that will predict the effect of the drug in the clinical situation. The purpose of bioassay is to ascertain the potency of a drug and to standardize the preparation so that each contains the uniform specified pharmacological activity. Thus, it serves as a pointer in the Commercial Production of drugs when chemical assays are not available or do not suffice and provides a predictive link towards quantitative pharmacology.

Keywords: Bioassay, potency, drug response, drug characterization, Pharmacopoea.

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INTRODUCTION

Bioassay or biological assay or biological standardization is defined as the estimation of the potency of an active principle in a unit quantity of preparation or detection and measurement of the concentration of the substance in a preparation using biological methods (i.e. observation of pharmacological effects on living tissues, microorganisms or immune cells or animal)[1]. It is used to study the effects of a new substance on a living body and is an essential step towards development of new drugs. Typically it involves three factors (i) stimulus, (ii) subject, (iii) response, i.e the change produced in the subject due to application of stimulus[2].

The history of biological assay dates back to primitive era where description of Noah's experiment from his ark by sending a dove repeatedly until it returns with an olive leaf. By this experiment Noah estimates the level of receding water from the Earth's grounds. The essence of this event, we find that it has all the three essential constituents of an assay, namely "stimulus" (depth of water), "subject" (the dove) and "response" (plucking of an olive leaf).

Serious scientific history of biological assay began at the close of 19th century with Ehrlich's investigation about standardization of diphtheria antitoxin. Since then, the standardization of materials by means of the reactions of living matter has become a common practice, not only in pharmacology, but in other branches of science also, such as plant pathology. However the assays were put on sound bases only since 1930's when some statisticians contributed with their refined methods to this area. The classic historical example of bioassay was the use of canaries by miners in past centuries as because canaries are more sensitive than human to noxious gases like methane. They reacted quickly to even small amounts of the gas. This would give the miners time to escape. At present, bioassays are used to analyze soil, freshwater and the sediment at the bottom of streams, rivers, saltwater and air. Therefore, the main objectives of bioassay are 1) characterization of the functionality of endogenous mediators, 2) pharmacological evaluation of new or chemically undefined substances, 3) Investigation of the side-effect profile, 4) monitoring environmental pollutants and assessing the pollutants quantity being released by wastewater or urban runoff[3]. Quantitative bioassays, are mostly used to determine the concentration of active principle of substances such as vitamin, hormone, and plant growth factor, whereas qualitative bioassays used to evaluate the physical effects of a substance which are not quantifiable, such as any abnormal development or deformity. An example of a qualitative bioassay includes Arnold Adolph Berthold's famous experiment on castrated chickens. This analysis suggested that by removing the testes of a chicken, it would not develop into a rooster, as because the endocrine signals necessary

for this process, were not available[2]. It is mostly used to analyze the activity of test drug which may be modified by adding other drugs and only acts in a certain way. For example, if a drug causes a piece of gut to contract and this effect is not blocked by Hexamethonium, which blocks ganglia, but is blocked by atropine, so the inference is that it is stimulating postganglionic acetylcholine receptors on the smooth muscle. Thus, qualitative bioassays are valid only when the specific drug block or enhance the action of test drug. Quantitative bioassay is only meant for investigation of the concentration and or potency of a substance by measuring its biological responses. Quantitative bioassay is typically analyzed through biostatistics methodology which is used to compare the activities of different drugs. For both theoretical and practical reasons it is necessary to distinguish between the assay methods for drugs which are agonists to stimulate a tissue, and those are antagonists to produce no effect themselves, but act by blocking the effect of agonists. So to quantify the degree of agonist and antagonist of a drug, it is very much essential to consider 'bioassay as a pharmaceutical tool' for any pharmaceutical preparation. Generally bioassays are of two types as mentioned below.

Quantal assay

A quantal assay involves an "all or none response"[2]. For example: Insulin induced hypoglycemic convulsion or the digitalis induced cardiac arrest. The drugs producing quantal effect can be assayed by end point method[1]. The response is either positive or negative as there is no intermediate response e.g. either convulsion occurs or doesn't. The quantal method is commonly employed for (1) Comparison of effective dose (ED_{50}) or median lethal dose (LD_{50}) and (2) Comparison of threshold response.

Graded assay

Graded assays are based on the facts that increase in the concentration or dose of a particular drug proportionately increases the observed response[2]. The drugs producing graded responses can be assayed by (1) Matching or bracketing method or (2) Graphical method[1]. This graded assay is based on the nature of the effect the substance that is expected to produce. For example, contraction of smooth muscle preparation for assaying histamine or the study of blood pressure response in case of adrenaline. The choice of graded assay depends on a) the precision of the assay required, b) the quantity of the sample available and c) the availability of the experimental animals.

Different Bioassay Techniques

End Point Method: Two groups of animals are taken (one receiving standard and other the test). The threshold dose of a particular drug is measured on each group of animals and then compared for the potency in the following way:

Conc. of Unknown = [Threshold dose of the Standard/ Threshold dose of the Test] × Concentration of Standard

Matching Method: In this method a constant dose of the test is bracketed by varying doses of standard till the exact match is obtained keeping the test sample at constant dose. Initially, two responses of the standard are those adjusted such that one is giving response of approximately 20% and other 70% of the maximum. The response of unknown which lies between two responses of standard dose is taken. The panel is repeated by increasing or decreasing the doses of standard till all three equal responses are obtained. The dose of test sample is kept constant. At the end, a response of the double dose of the standard and test which match each other are taken. These should give equal responses. Concentration of the test sample can be determined as follows:

Conc. of Unknown = [Dose of the Standard / Dose of the Test] X Conc. of Standard.

The main constraints of this method are:

1. It occupies a larger area of the drum for the tracings.
2. The match is purely subjective, so there are chances of error.
3. This method does not explore the dose-response relationship.

However, this method is particularly useful if the sensitivity of the preparation is unstable.

Graphical method: This method is based on the assumption of the dose-response relationship of the graded doses of the standard and two equiactive responses of the test sample. The height of contraction is measured and plotted against the log-dose. The dose of standard producing the same response as produced by the test is read directly from the graph and the concentration of test sample is determined by the same formula as mentioned before. The characteristic of log-dose response curve is that it is linear in the middle (20-80%). Thus, the comparison should be done within this range only. In other words, Log-dose-response curve is plotted and the dose of standard producing the same response as that of produced by the test sample is directly read from the graph. In simpler design, 5-6 responses of test sample must lie within this range. Advantage of this method is that, it is simple and chances of errors are less if the sensitivity of the preparation is unchanged. This method is used for bioassay of antagonist. The responses are determined in form of the percentage inhibition of the fixed dose of agonist, which are then plotted against the log dose of the antagonist and the concentration of unknown is determined by examining the amount of the standard producing the same effect as produced by the test[1]. The activities of different agonists can be expressed as the ratio of the concentrations which produce the same response. If *a* molar solution of compound A produces the same effect as that of *b* molar solution of compound B, the molar activity of B relative to A is *a/b*; if *a* is 10^{-3} M and *b* is 10^{-2}M, then, the weaker

compound has one tenth of the activity of the other compound. These assay techniques can be adapted for obtaining the relative activities or equipotent molar ratio, of different agonists.

Bioassay of Some Important Drugs

Depending upon pharmacological action of various drugs, different preparations may be used. Table 1 gives different preparations and the pharmacological activity for which a particular drug is assayed:

Table 1. Drugs with their isolated tissue preparation and the pharmacological activity assayed against it[1].

Drug	Preparation	Activity Assayed
Digitalis	Cat blood pressure	Fall in B.P. and death
	Pigeon	Emesis
	Guinea pig blood	Stopage of heart pressure and death.
Adrenaline	Blood pressure of the spinal cat	Rise in B.P.
	Isolated rabbit duodenum, Isolated rat uterus, Isolated caecum of fowl.	Inhibition of the tone
Noradrenaline	Blood pressure of the pithed cat	Rise in B.P.
Acetylcholine	Isolated rectum abdominis muscle of frog, Rat ileum, Leech dorsal muscle	Contractile effect
	Rat/Cat blood pressure	Fall in blood pressure
	Isolated mouse heart	Inhibition of cardiac Contractions
Histamine	Isolated, atropinized terminal ileum of guinea pig	Contractile effect
	Anaesthetized and atropinized cat.	Fall in blood pressure
5 Hydroxy-tryptamine	Isolated atropinized rat uterus, Isolated terminal colon of rat, Isolated fundus strip of rat stomach	Contractile effect
	Perfused rabbit ear	Constriction of blood vessels
Curariform drugs.	Rabbit	Dropping of head
e.g. d-tubo-curarine	Rat diaphragm with curarine phrenic nerve or Cat gastrocnemius muscle with sciatic nerve.	Inhibition of the contractile effect
Heparin	Whole blood of ox with thrombokinase extract and acetone dried ox brain	Prolongation of blood clotting time
Antibiotics	Suitable micro-organism grown on nutrient agar medium	Inhibition of growth of suitable micro-organism
Vitamin D	Rats maintained on richetogenic diet stage.	Alleviation of rachetic
Insulin	Rabbits	Lowering of blood-sugar Level.
	Mice	Convulsions and/or death due to hypoglycaemia
	Isolated rat diaphragm	Increase in glycogen content.
	Rat's epididymal fat	Increased metabolism of glucose, indicated by increased in CO_2 production.
Oxytocin	Adult cockerel	Vasodepressor activity
	Isolated rat uterus	Contractile effect

	Rabbits (female)	Ejection of milk from mammary duct.
Vasopressin	Rat blood-pressure	Vasopressor activity
Growth hormone	Hypophysectomized rats.	Gain in weight. Increase in width of epiphyseal cartilage
Gonadotrophin (FSH)	Hypophysectomized male rats	Increase in testicular weight.
	Hypophysectomized female rats	Increase in weight of ovaries.
Gonadotrophin (L.H.)	Immature male rats	Enlargement of prostate gland.
Gonadotrophin (FSH and LH)	Immature female rats	Increase in weight of uterus.
Prolactin	Cloves of pigeons	Increase in weight of crop sac.
	Female guinea pig or rabbit.	Secretory changes in mammary gland.
	Female rats	Lengthening of estrous cycle and function of corpus luteum
	Hypophysectomized rat	Inhibition of estrogen upon vaginal smear.
* Corticortrophin	Hypophysectomized rats	Depletion of ascorbic acid from adrenal gland.
* Thyrotropin	Mice or rats	Release of previously administered ^{131}I (Iodine) from thyroid gland
* Androgen	Castrated capon	Increase in size of comb.
	Castrated male rat	Increase in weight of prostate gland and seminal vesicles.
	Castrated male rats	Increase in weight of levator-ani muscles
Estrogen	Rat or mouse	Increase in weight of (Female) uterus
Progesterone	Sexual immature rabbits	Proliferative changes in endometrium of uterus. Increase in carbonic anhydrase-activity in uterus

*Radioimmunoassay or radio receptor assay methods are also available[1].

Although bioassays are beneficial in determining the biological activity within an organism, they can often be time-consuming and laborious. Organism-specific factors may result in data that is not applicable to others in that species. For these reasons, other biological techniques are often employed, including radioimmunoassays.

Environmental Bioassays

Environmental bioassays are generally the burning topic of interest and are used for broad-range survey of toxicity. United States conduct certain bioassays procedures for industrial dischargers and municipal sewage treatment plants called whole effluent toxicity tests to control water pollution. This is done by exposing living aquatic organisms to samples of industrial waste water [3,4]. The main experimental animals used for these bioassays of aquatic samples are fathead

minnows, earthworms, various aquatic invertebrates, lettuce seeds and protozoans[5]. Several studies have compared the sensitivities of various types of seeds to common pollutants[6]. The following are a few examples of ways in which lettuce seed bioassays have been used by scientists for environmental testing purposes:

1. To map areas for clean-up of Superfund sites[7]
2. To screen industrial effluents[6]
3. To test the effectiveness of clean-up of lead-contaminated soil[8]
4. To design clean-up strategies at a site contaminated by treatment of lumber with creosote and other compounds[9]

CONCLUSION

The different bioassays are routinely used in various academic institutions and pharmaceutical industries. With the advent of technology, availability of advanced, sophisticated and more reliable analytical methods the scenario for bioassay has changed dramatically and are being replaced by other chemical assays like Titrimetric method, UV absorbance, Chromatography and HPTLC. Titrimetric method is the recommended assay method for Acetylcholine, Thyroxine and Heparin; UV absorbance for Adrenaline, Progesterone and Digoxin; Chromatography for Estradiol and Penicillin; HPLC for Insulin respectively [10]. The main problem with all types of bioassay is that of biological variation. However, the current technological advancement have led to the design of bioassays in minimizing variation, avoiding systematic errors resulting from variation, estimation of the limits of error of the assay result.

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