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VISUAL EXPERIMENT

Isolation of mouse internal organs for molecular and histopathological studies

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ABSTRACT

Since auto-immune diseases dramatically affect function of various tissues and organs *in vivo*, each integral organ of mouse has significant value in order to analyze the effect of any pharmaceuticals or test drugs to confirm their protective efficacy. Hence, this study reports step-by-step visual demonstration of isolation of various internal tissue organs such as liver, spleen, adipose tissue, muscles, and pancreas from mouse model which have significant contribution to design in-depth molecular level studies along with histomorphological evidences that may assist research to set innovative results at molecular level, thus may help to prove the efficacy of any test sample as a new drug of pharmaceutical importance.

INTRODUCTION

From time immemorial, man has depended on animals for his survival, either as food or for competition and companionship. We have come a long way since then and specially bred laboratory animals consisting of mice, rats, hamsters, guinea pigs, rabbits, cats, dogs, monkeys, higher farm animals and a variety of birds and other lower forms are now integral part of biomedical research (Haenen et al., 2014). *In vitro* assays typically rely on simple interactions of chemicals with a drug target, such as receptor binding or enzyme activity inhibition. However, *in vitro* results often poorly correlate with in vivo results because the complicated physiological environment is absent in the *in vitro* testing system. Although cell-based assays can provide some information, cultured cells still do not provide physiological conditions and complex interactions among different cell types and tissues. Moreover, cell lines are usually transformed, exhibiting different gene expression and cell cycle profiles than those of cells in the living organism.

There is a growing trend of using animal tissues for drug discovery research (Deng et al., 2016). Research, in reality, involves three facets: a) acquisition of new knowledge, b) use of animals in teaching exercises, and c) testing of compounds, chemicals or devices for safety and effectiveness. There must be reasonable expectation that research involving animals will contribute significantly to present and future knowledge, which may eventually lead to the protection and improvement of the health and welfare of either humans or animals. World over, new drug research as well as tests meant for assuring the quality and efficacy of pharmaceutical products /vaccines/biological are based on experiments involving animals. Toxicological studies especially those performed in rodents are the essential link between the pre-clinical phase and clinical development of the drug molecule. No new drug can be introduced in clinical practice or even for the matters into clinical research unless it passes the battery of toxicity tests in animals (Bajpai et al., 2016).

Researchers have developed animal model systems using both vertebrates and invertebrates for drug screening. Thus, this study reports visual demonstration of isolation of various internal tissue organs from mouse model which may contribute significantly to design in-depth studies for molecular biologists. The small size, high fecundity, and experimental tractability of these animals enable cost-effective and rapid screening of numerous compounds.

MATERIALS AND EQUIPMENTS

- Mouse
- Dissection tray
- Forceps
- Scissors
- Surgical spatula
- Surgical gloves
- Face mask (if necessary)
- 70% ethanol
- Phosphate buffer saline
- Petri dishes
- Freezer (-80˚C) for preservation of animal tissue slides until further analysis

PREPARATION OF REAGENTS

Phosphate buffer solution 1x (pH 7.4)

NaCl	8 g
KCl	0.2 g
Na_2HPO_4	1.44 g
KH_2PO_4	0.24 g

Make volume 1 liter and mix well and autoclave. Use 1M HCl to adjust the pH if needed, and keep at room temperature.

10% Neutral buffered formalin

37% Formaldehyde	50 mL
Distilled water	450 mL
Na_2HPO_4 (dibasic)	3.25 g
NaH_2PO_4 (monobasic)	2.00 g

Make volume 500 mL, mix well and keep at 4°C.

Trizol reagent

Ready to use and purchased from Sigma-Aldrich (USA)

VIDEO CLIP

Isolation of organs: 7 min 55 sec

METHOD

Dissection and isolation of internal tissue organs from mouse

1. Collect anesthetized mouse and bring on the dissection bench
2. Clean your dissection bench with 70% ethanol to avoid any cross contamination
3. Make sure you have all necessary equipments needed for dissection
4. Pin or tape the legs of mouse to the dissection board

5. Begin the dissection starting from the initial incision below the navel going up toward the mouth. Be careful not to cut through the membrane
6. Make a downward incision toward the tail to allow the skin to be opened
7. Make an incision laterally at the shoulder joints to allow the skin to be peeled open
8. Repeat the lateral incision at the pelvic girdle and use forceps and fingers to peel back the skin layers.
9. Remove the abdominal membrane using scissor and forcep gently to reveal the internal organs
10. Cut through the sternum (the bone at the front of the ribcage) and expose the skin to the tape board side
11. Make identification of organs for gentle separation and isolation using scissor and forcep and keep each isolated organ separately in petri dishes already filled with sterilized phosphate buffer saline

Steps in tissue organ isolation and preservation

Tissue organ isolation

1. During isolation of organ tissue samples, they should be washed using phosphate buffer saline to remove the traces of blood and other contaminants. Washing steps should be performed at room temperature

Preservation of tissues for histomorphological analysis

1. A 10% solution of neutral buffered formalin is referred as a tissue preservation medium for histomorphological analysis. Samples should be stored at -80°C

Preservation of tissues for molecular level studies

1. Ready to use Trizol™ reagent should be used for long-term preservation of tissue organ samples which can be further analyzed for mRNA extraction and cDNA preparation during molecular level studies. Samples should be stored at -80°C

DISCUSSION

This is necessarily to be undertaken as to unravel the secrets of nature. If we essentially know how different tissues and organs are kept healthy, we can then find out what goes wrong when disease strikes. To conquer disease, a lot of work needs to be put into, by way of developing better medicines, perfecting surgical operations as well as making vaccines and finding other ways of preventing diseases. There are many diseases which are yet to have a proper cure like multiple sclerosis, certain cancers, diabetes as well as new diseases like AIDS, and Alzheimer disease (Bajpai et al., 2016). All these need initial input in terms of animal experiments.

A wide range of chemicals and medicines which are used in day-to-day life, as household products, in farming, industry etc., need to be tested for their safe use in humans as well as in animals. Such preliminary testing is very much essential for avoiding pollution and associated health hazards and proper healthy maintenance of the environment. There are many diseases which are inherited fully or partially and are caused by basic faults in a person's genetic code. Some of the animals also have similar genetic fault as humans do. Hence, study of specific biomarkers associated with specific diseases associated with specific tissue organs (liver, spleen, pancreas, spleen and muscle) can reveal innovative research to improve human and animal welfare. The animal thus plays a vital role in understanding and treatment of such genetic diseases. Scientists have now made such progress in molecular biology that they can now alter genes and breed strains of mice and other animals with particular genetic diseases. This may ultimately lead to treatments in genetic disorders like cystic fibrosis, sickle cell anemia and other diseases which run in families. If the treatment of a disease is to be effective, an accurate and quick diagnosis is essential. Animal experiments in terms of effectiveness of any test known or unknown drug are vital in this area. Animal tests have paved the way for many blood tests for the diagnosis of infectious diseases.

Based on the above study, it can be concluded that visual demonstration of dissecting animals and/or isolating their integral tissue organs can be of great significance in teaching pharmacology and medicinal biology students and researchers to understand the basic anatomy and physiology of man and animals at biochemical and molecular levels.

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PRECAUTION

During dissection, care should be taken not to cut any internal organ tissue to avoid blood streaming.

After washing the tissue samples, they should be maintained on ice to maintain the integrity of tissue organs as it might cause variations in the results.

A proper selection of preservative medium should be made according to the studies to be performed further.

Care should be taken while storing the tissue at proper temperature range for short-time as well as for long-time preservation.