LUPINE PUBLISHERS

Open Access Journal of Complementary & Alternative Medicine

DOI: 10.32474/OAJCAM.2020.02.000135

ISSN: 2644-1217

Research Article

Experimental Animal Model for Acute Toxicity Testing with Natural Products

Emerson Barbosa da Silva*, Patrick Gabriel dos Santos Pessini, Camila dos Santos Chagas, Daniel Santos Neves, Rodrigo Pereira Barbosa, Giuliana petri and Fernando Luiz Affonso Fonseca

University Health Center ABC , Lauro Gomes Avenue, Brazil

***Corresponding author:** University Health Center ABC-Lauro Gomes Avenue, 2000-Vila Sacadura Cabral- Santo André-SP -Zip Code: 09060-870, Brazil

Received: January 20, 2020 **Published:** February 11, 2020

Abstract

BALB/c mice are often used in laboratory researches and its methodology needs to be standardized. A number of mice previously inoculated with Ehrlich's tumor were used under appropriate conditions to collect blood samples and analyze hematological parameters in automated and semi-automated equipment and allow this methodology to be made. The study successfully defined and established the methodology needed to obtain reference values for hematological parameters and to assist researchers develop their own methodology about animal handling and group management in experiments.

Keywords: Ehrlich tumor carcinoma; Experimental animal model toxicity

Introduction

The use of animals in scientific research is extremely important, especially when we refer to experimental research. Animals are used to improve understanding of physiological, metabolic mechanisms and also to discover new tests, improving existing tests and drugs used in human and veterinary medicine assisting in scientific development. [1]. It is then recognized that animal research has had a major impact on longevity and improved human and animal welfare, highlighting advances related to organ transplants, vaccine production, cancer research, cardiovascular disease treatments, drug production and control [2].

Experimental animals must be kept in a strictly controlled environment that meets the parameters of sanitary and genetic quality to correctly obtain results with minimal interference in order to guarantee the quality and reliability of the applied tests and data obtained. [3]. Many species of animals have been used in research, as the mouse being the most used animal for researches related to human pathologies, because the mouse has some favorable characteristics: physiological similarities with humans, small size, short gestation period, easy maintenance and handling [1].

More than 90% of scientific research currently uses mice from isogenic strains. The isogenic mouse is obtained by mating at least 20 inbreeding generations from a single couple, generating an inbreeding coefficient of 98.6% and isogenic strains have great value because they allow experiments to be carried out eliminating the factors of genetic variability, allowing the use of fewer animals to achieve the necessary statistical power [4].

A wealth of information about isogenic mice can be found, such as: genetic mapping, histocompatibility parameters, physiological, pathological and immunological parameters that are mainly useful for cancer research and transplantable tumors for therapeutic drug testing. BALB/c mice are isogenic, presenting low incidence of ovarian cysts and mammary tumors, with zero incidence in male animals, 5% in pregnant females and 1% in virgin females. Despite that, BALB/c easily develops other types of cancers, including reticular neoplasms, lung tumors, and kidney tumors [5 & 6].

Ehrlich's tumor is a type of transplantable experimental neoplasm, species-specific, of malignant epithelial origin, corresponding to the female mouse mammary adenocarcinoma; the tumor develops in different strains of animal species in ascitic

Copyright © All rights are reserved by Emerson Barbosa da Silva.

form when inoculated via intraperitoneal, and in solid form when inoculated subcutaneously and because of its high invasiveness rate, the Ehrlich's tumor is frequently used to study the action of physical, chemical and biological compounds in experiments regarding cell pathogenesis, immunology, cytogenetics and even therapy [7-9].

It is essential to use laboratory tests to individually evaluate changes in the physiological and functional profile of the animal. In experimental procedures where pathological processes are induced, the knowledge of normal reference values is indispensable, since some pathologies influence the metabolism and alter the tests results, and from these results can evaluate the degree of disease and effectiveness of treatments [1,10,11].

The hematological and biochemical parameters provide important information about the clinical conditions of animals, nutritional status, presence or absence of infections and allow monitoring of tests and prognosis and each vivarium should have the dedication to define laboratory parameters and reference values for the tests used in its animals [12].

Method

Animals and ethical aspects

All experimental procedures described in this study must approved by the local Animal Experimentation Ethics Committee. The experimental groups need an average of 6 animals by group of albino mice, BALB/c strain, with an average weight of 30±5 grams. During the experiment, the animals needs to be kept in a 12-hour light/dark photoperiodic cycle with controlled ventilation (20 air changes/hour), temperature and relative humidity between 45 and 65%, being fed with filtered water and animal feed offered ad libitum maintained for 28 days. The animals need to be handled quickly and carefully through the base of the tail, following the protocol of good animal care practices from the local vivarium [13].

Experimental design

To obtain the solid tumor, tumor cells must be obtained from the Ehrlich's ascites tumor with seven days of evolution. The number of total cells that will be subsequently implanted must be determined by counting under optical microscopy with the aid of a Neubauer chamber. Ascitic fluid need to be diluted ten times with saline and aliquoted. A cell viability test must be performed with Trypan-Blue dye, considering viable only suspensions with cell viability greater than 95% according to the protocol of the Clinical Analysis Laboratory from the local University. The animals must be sheared in the dorsal region of the mouse, and the cell suspension inserted by injection with a 24-gauge needle, at a concentration of 2.105 cells/mL in the lateral region of the back of each animal.[14].

After 7 days of inoculation, the researcher will begin the observation of tumor progression in size (cm^2), animal weight and survival of the animal during the maximum period of 28 days. The animals must be divided into three groups for analysis: 7, 21 and 28-days for the purpose of obtaining results, allowing the comprehension of laboratory parameters not only after 28-days but throughout the tumor development to evaluate the disease progression to provide parameters to future researches that will evaluate the same tumor in acute and chronic phases.

Blood collection procedure

After 7, 21 and 28 days of evaluation, respectively, the mice will be euthanized by intraperitoneal sodium thiopental or drug with similar pharmacological action as alternative anesthetic overdose (100mg/kg). After entering the anesthetic plane, the laparotomy is to be performed and then the caudal vena cava puncture, through which the largest amount of blood will be removed leading to the animal's exsanguination. The blood will be homogenized and conditioned in pediatric plastic tubes containing the anticoagulant K2EDTA and after collection the blood will be transported to the laboratory to be analyzed using automated hematological analysis. [15]

Hematological analysis

Hematological determination will be performed by flow cytometry method, automated equipment being performed the erythrogram, leukogram and platelet parameters, and the microscopic analysis of the blood smear to perform the hematological cell differentiation, following the rules of good practice in clinical laboratory analysis.

Statistical analysis

For qualitative variables, absolute and relative values need to be used. To express the non-normal quantitative data (Shapiro-Wilk < 0.05) will be used the median, 95% confidence interval and 25 and 75 percentiles. For the data that presented normality (Shapiro-Wilk > 0.05), will be used: mean, standard deviation, minimum and maximum. In the association of the groups with the markers, total score, animal weight, tumor weight and markers, the chi-square, Kruskal-Wallis and ANOVA tests needs to be performed. And for all analyzes a 95% confidence level needs to be considered. Then use statistical analysis software if needed.

Conclusion

It is concluded that this work successfully defined the appropriate methodology regarding ethical animal handling and hematology reference values, supplementing future researches and helping other researchers interested in using the BALB/c animal model in Ehrlich's tumor perspectives in toxicity and myelotoxicity assays using natural products.

References

1. Santos E, Cunha de Oliveira D, Hastreiter A (2016) Hematological and biochemical reference values for C57BL/6, Swiss Webster and BALB/c mice. Braz J Vet Res Anim Sci 53(2): 138-145.

2. Ferdowsian HR, Gluck JP (2015) The Ethical Challenges of Animal Research: Honoring Henry Beecher's Approach to Moral Problems. Cambridge Quarterly of Healthcare Ethics 24(4): 391-406.
3. Barbee RW, Turner PV (2019) Incorporating Laboratory Animal Science into Responsible Biomedical Research. ILAR J.
4. Casellas J (2011) Inbred mouse strains and genetic stability: a review. Animal 5(1): 1-7.
5. Hoag WG (1963) Spontaneous cancer in mice. Ann N Y Acad Sci108: 805-831.
6. Cheville NF (1988) Introduction to veterinary pathology.
7. Tannock IF (1969) A comparison of cell proliferation parameters in solid and ascites Ehrlich tumors. Cancer Res 29(8): 1527-34.
8. Palermo-Neto J, de Oliveira Massoco C, Robespierre de Souza W (2003) Effects of physical and psychological stressors on behavior, macrophage activity, and Ehrlich tumor growth. Brain Behav Immun 17(1): 43-54.
9. Silva AE, Santos G, Cassali G (2006) Marcadores de proliferação celular na avaliação do crescimento do tumor sólido e ascítico de Ehrlich. Arq Bras Med Vet Zoo tec 58(4): 658-661.
10. Davies B, Morris T (1993) Physiological parameters in laboratory animals and humans. Pharmaceutical research 10(7): 1093-1095.
11. Fernandes DP, Pimentel MML, Santos FAD, Praxedes ÉA, Brito PD, et al. (2018) Hematological and biochemical profile of BALB/c nude and C57BL/6 SCID female mice after ovarian xenograft. An Acad Bras Cienc 90(4): 3941-3948.
12. Schnell MA, Hardy C, Hawley M, Propert KJ, Wilson JM (2002) Effect of blood collection technique in mice on clinical pathology parameters. Hum Gene Ther 13(1): 155-161.
13. Da Silva EB (2019) *Synadenium Umbellatum* and the Ehrlich's solid tumor treatment: Assessment of inflammatory regulators (transforming growth factor protein-β1 and tumor necrosis factor-α) gene expression, hepatotoxicity and myelotoxicity. European Journal of Oncology Pharmacy 2(1): 9-13.
14. Da Silva EB (2019) Comparative Study on Myelotoxic and Antineoplastic Action of *Synadenium umbellatum*, *Vitis vinifera* and Resveratrol. Journal of Natural Remedies 19(2): 80-86.
15. Pessini PGS (2020) Hematological reference values and animal welfare parameters of BALB/C-FMABC (*Mus musculus*) inoculated with Ehrlich tumor kept in the vivarium at ABC Medical School. Animal Models and Experimental Medicine.

This work is licensed under Creative Commons Attribution 4.0 License

To Submit Your Article Click Here: Submit Article

DOI: 10.32474/OAJCAM.2020.02.000135

Open Access Journal of Complementary & Alternative Medicine

Assets of Publishing with us

- Global archiving of articles
- Immediate, unrestricted online access
- Rigorous Peer Review Process
- Authors Retain Copyrights
- Unique DOI for all articles