The Lab Rats

The Role of Animal Testing in Cancer Research

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Abstract

Animal testing was first used by Aristotle and Erasistratus in early Greece. They used animal experiments to advance the general understanding of pharmacology, anatomy, and physiology.[1] Our aim in researching this topic was to deepen our knowledge of how animal testing works, its role in cancer research, and how it will improve in the future. We used the databases PubMed, NCBI, the Mayo Clinic, and AnimalResearch.info to research. Using these databases, we found that animal testing plays a big role in cancer research, and has made many improvements and discoveries since the Greek began using it. Today, many new technologies are being used to help find a cure for cancer.

Why do scientists test on animals (*in vivo*)?

- Animal testing allows scientists to generate tumors in the definitive areas and check on the tumor growth pattern and metastasis post experimental therapeutic treatments run on the animals. The effects of treatment can also be tested by isolating the cancer cells and running different experiments, which means more accurate testing.[2]
- It is useful when finding side effects in the entire body instead of just one part.[4]
- Animals suffer from the same illnesses as humans do, with much of the same symptoms.[4]
- Animals can be used to test treatments for diseases, protecting humans and animals.[4]

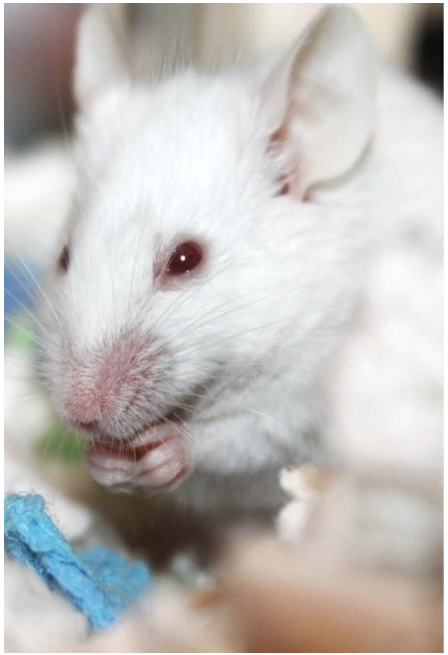


Photo by Nature_Blossom via https://pixabay.com/photos/rodent-cute-mammal-little-animal-3177373/

Why are 95% of animals tested on mice and rats?

- They are easy to take care of.[3]
- They have relatively short lifespans of 2 to 3 years, allowing generations of mice to be observed in a short period of time.[3]
- They can be handled easily by researchers because they are docile and obedient.[3]
- The mice and rats used in testing are bred so that they are genetically identical, allowing experiments to be uniform and more accurate.
- The anatomy of rats and mice are well understood by researchers, making it easier to understand changes in behavior.
- Mice and rats are very similar to humans anatomically.

What is the difference between mouse and human models?

Mouse Models

- There is only 9 weeks between generations.[2]
- Easier to manipulate.[2]
- Smaller in size, causing slight differences, such as the amount of medicine or tumor injected, resulting in minor implications.[5]
- Can be bred uniformly, isolating the tumor, and increasing accuracy.[3]
- 90.5% of dangerous drugs have been kept out of clinical trials thanks to animal safety tests. This helps eliminate drugs that are ineffective without harming humans.[7]

Human Models

- Though it is not allowed to test on humans, in clinical trials, people can voluntarily participate to help see a drugs' effects.[2]
- Most accurate[2]
- We can see the actual result of certain drugs and their effects on the human body.

How Cancer Medicine is Found

- Many tests are done to find if different cancer medicine works. Scientists test on plants, animals, fungi, and in the end, humans to run the approved drugs in clinical trials.[14]
- Scientists compare cancer cells with healthy cells. They figure out the difference between the two and focus on what to do to change medicine in order to target these differences.[14]

  Example: many drugs target cdk4 proteins. These are called cdk4 inhibitors and they try to stop the cdk4 protein from forming into cancerous tumors.[14]

<u>Types of Cancer Treatment:</u>

- Chemotherapy: the use of drugs to kill cancer cells. This therapy tries to kill the cancer cells but can end up killing fast growing healthier cells which leads to hair loss and many other side effects.[17]
- Radiation: this therapy is the use of intense electromagnetic waves on your body to try and kill off the cancer cells inside.[17]

Regulatory Forces and Animal Testing Abuse

To have any type of animal testing, the IACUC (Institutional Animal Care and Use Committee) has to review every proposed protocol. The IACUC guides the care of test subjects. They have the power to deny or approve any proposed action. This regulatory force board ensures that no cruel or harmful act is done to any animals being tested on.[12]

Each proposed protocol must include:

- Justification for using animals, how many animals to be used, and the species chosen.
- A description of sources and methods used to search for an alternative to painful procedures. (procedures and drugs used to induce minimal pain/discomfort)
- Description of the study to ensure that the experiment does not duplicate a previous research.[12]

Animal Testing Regulations in Foreign Countries

UK	There are proper controls in UK for Animal Testing, it is required to have medicine tested on animals before given to humans.[8]	• Researchers must provide proper outline of testing and proof of no alternative.[8] • 3 R's: Reduce Animals, Replace with Alternatives, Refine Animal Testing.[8]
South America & Brazil	Animal Testing Laws are recently coming in as Brazil has a large animal population.[9] Few South American countries have animal testing laws.[9]	• Arouca Law prevents testing on vertebrate animals.[9] • First regulation published in 1985 : COBEA Ethical Principles.[9]
China	Many of China's laws still need improvement. The laws are not focused on animal protection, more on production. [9]	• Ministry of Science and Technology oversee testing but are not as strict as needed.[9] • Cruelty is common & large population makes laws hard to abide by.[9]

Engineered Mice Models

Engineered Mice Models are types of mice that are bred in a lab to help research.[2] They help scientists further their research in testing and they are very easy to breed.[2] There are different types of Engineered Mice Models including: transgenic models, somatic engineered models, genetically engineered models, and transplantation models .[2]

***Transgenic Mice Model*: A mouse model where there is random implantation of tumor-causing DNA in a mouse genome. These are usually bred in a lab and produce quicker than other testing methods but also are rather cheap in terms of money.[2]**

***Somatic Engineering Model:* When specific sites in a mouse model is targeted and manipulated, often to see the effects of cancer or to simulate cancer.[2]**

Engineered Mice Models (Continued)

Genetically Engineered Model: When a mouse is bred in a lab and modified to have a specific tumor or have a malfunction to be used for further cancer testing. These sites are usually manipulated so that they can be tested on and used to find a cure.[2]

Photo by sibya, via https://pixabay.com/photos/mouse-rodent-rat-mice-pest-3194768/

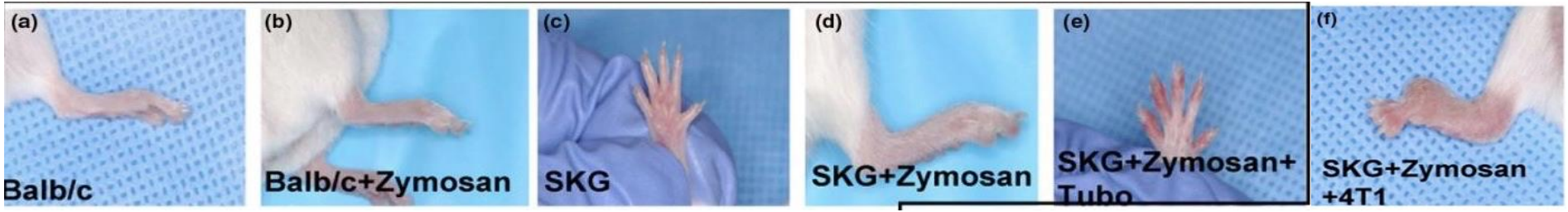
Transplantation Model: When a mouse model is injected with an infection or tumor that is common in humans in a certain site.

- Orthotopic: When infection or tumor is inserted into a similar or comparable part of the body of the mouse model as to the original site of tumor.
- Ectopic: Put in a different part of the body instead of the original site of the tumor.[2]

Transgenic Mice (Use in Lab)

- An example of the use of Transgenic Mice is when a laboratory tested for the relation of breast cancer to autoimmune arthritis, commonly found in humans.[7] Places of inflammation and diseases as such have commonly been sites of cancer tumors, but never actually tested.[7]
- In this test, transgenic mice that have been genetically coded to have autoimmune arthritis, were bred. They were inserted with certain trigger chemicals that provoked inflammation.[7]
- As a result, these researchers were able to prove that people with breast cancer that also have autoimmune arthritis also are more likely to have metastatic cells in other parts of the body. This means they have a higher chance of bone destruction and cancer cells spreading through the body.[7]

"Induction of arthritis in SKG mice. Images of the hind and fore limbs."by Das, Roy et al. is licensed under BioMed Central Ltd.



Modern Technologies in Animal Testing

Cre/LoxP System: This modern technology is, put simply, a cutting tool for DNA. This tool allows researchers to perform targeted knockouts and perform inversions, deletions, and translocations.[11] A Cre/Lox P helps researchers target a certain gene and do gene recombinase (an enzyme used to help genetic recombination).[11] Cre is an enzyme whereas LoxP is the site where there are palindromic repeats.[11] This is the place to perform site-specific Cre recombinase.[11]

Inversion: Flipping the order of the DNA[10]
Deletion: Removing the Targeted Gene[10]
Translocation: Moving the targeted gene somewhere else.[10]

Cre/LoxP can also help with conventional knockouts, which are a precursor to conditional knockouts. Conventional knockouts are when a targeted gene is removed in the whole body, whereas conditional knockouts (more recent) take out the targeted gene in a specific site.[10]

Process of Forming Transgenic Mice

Method 1 (Embryonic Stem Cells)

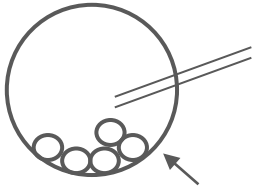
Embryonic Stem cells are the stems of unborn embryos. [6]

ES Cells

The ES Cells that express the certain gene are picked up in a vector.[6]

Vector

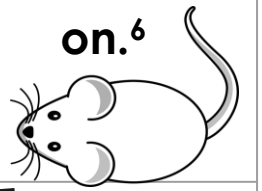
ES Cells are injected into cell mass.[6]



Inner Cell Mass

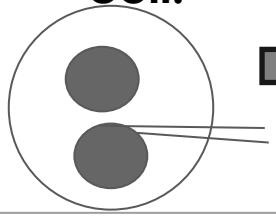
The fertilized egg (zygote) is placed in the uterus of a surrogate mother.[6]

Mice are bred to show certain genes and be tested on.[6]



Method 2 (Inserting Gene)

Desired gene is put in egg cell.[6]



Fertilized egg cell with pronuclei is implanted in uterus of foster mother.[6]

The gene must be inserted before the sperm fuses with the female pronucleus, forming a zygote.[6]

Offspring is tested for presence of the gene.[6]

Diagram created by the Lab Rats

What is going on NOW with Cancer Research?

Cancer diagnosis became increasingly improved with innovations in software and data science (tested on animals). These innovations are continuously being improved through testing. New innovations in cancer research include:

{The Auris™ Monarch™} - a new robotic platform to fight against lung cancer. It combines software, data science, robotics, and endoscopic innovations. With these innovations, diagnosing has less complications and are more accurate.[15]

Liquid biopsy - a new way to monitor cancer patients. Most tumors shed CTCs (Circulating Tumor Cells) into the bloodstream during cancer progression. The liquid biopsy analyzes the CTCs shed. Monitoring the circulating tumor cells helps predict the response to therapy and tumor recurrence without invasive and discomforting procedures.[15] Although this is true, many times the sensitivity of the liquid biopsy isn't high enough and more testing is being done to improve its accuracy and ensure that it is effective.[15]

Immunotherapy

Immunotherapy - an advanced cancer treatment that dramatically changed cancer management. Animal testing played a key role in the development of these immunotherapy techniques.[15]

Immunotherapy often gives the body man-made immune system proteins to become stronger. Immunotherapy uses drugs that release the brakes of the immune system. With these medicines, the body can destroy cancer cells. Once the body can respond to cancer cells, it can slow or stop cancer growth. Examples: nivolumab (Opdivo®), pembrolizumab (Keytruda®).[16] These drugs are still being tested on to decrease its toxicity and researchers are still working on to ensure that it does not become a complication.[16]

These drugs along with chemotherapy or by themselves resulted in longer survival and tumor progression free survival.[16] Animal Testing has played a crucial role in discovering these therapies. Before being given to humans, it has to work on animals and testing has to prove its safety.

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