Can animal testing be replaced by non-animal models?

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Today many new techniques are being developed with the purpose of replacing animal testing. The benefits of these new methods are many, there are the ethical winnings, economical and practical advantages and the fact that the non-animal models can give better results, leading to for an example safer pharmaceuticals When an animal, like a rat or a mouse, is asked to predicts the effects of a substance in humans they are right almost as often as they are wrong. But the disadvantages of the new non-animal models are also many.

"The opportunity to understand and treat disease of humans would increase enormously if the scientists would devolve away from animal testing and on to studying human blood and tissues"
Mark Davis, director of the Stanford institute for immunity, my translation from the homepage of the Swedish Fund for Research Without Animal Experiments.

The top picture shows a common laboratory rat. The bottom picture shows a cell, in which the DNA has be colored green. The big green areas are two cell nuclei and the small green dot is the micronucleus.

Animal models do not always give the same predictions as cell models

Before a new pharmaceutical is to be released on the market, we need to know if there is any risk it will hurt our DNA, if it does it might induce tumors and hereditary diseases. To find out the risk of possible DNA damage one well used technique is called the micronucleus test. This method could be used in animals (in vivo) by giving the animals the test substance, wait a few hours, days or weeks, and then kill the animal and study its cells in microscope. The same test could also be done outside the animal (in vitro), by exposing only cells to the test substance, wait and then study them in microscope to see if any harm is done. But, as similar as the two approaches are, the in vitro and the in vivo do not always show the same results. This is what happened when scientists tried to find out if treatment with the female sex hormone estradiol could hurt our DNA. The scientists had been using the micronucleus test, and in animals the hormone seemed to be harmless to the DNA. Exposing cells outside the animal, however, gave great DNA damages. Way is this?

Terminology

In vivo – in living animals
In vitro – in glass, outside the animal

In vitro models could give very detailed information

One of the great opportunities of in vitro models is that one can use human cells, and through that get a better prediction of what the effects would be in a human, in contrast to using for example a mouse or a rat. There are major differences between humans and commonly used experimental species. In many ways other animals do not respond to chemicals in the same way as we do, their physiology is different. Also when used in pharmaceutical studies animals are often induced with artificial diseases that are similar but not the same as the disease in humans.

When doing an experiment in vitro it is much easier to determine the exact amount of a chemical that reaches the cells, and trough that determine exact doses that are harmful or not. In vitro you can also more exact determine the amount of time the substance is present at the cells. In vitro experiments are generally shorter than in vivo, it is easier to keep an animal alive for long then it is to keep cells. This is both good and bad. The effects of estradiol for example fade after a few hours, and if the experiment goes on to long the effect will not be visible any more. Of course for some other chemicals the relationship is the opposite, it takes long time for it to give harm and then using cells could be a problem.

By using in vitro models we can design experiments more in detail than what is possible in animal experiments. It gives us an opportunity to study human cells, from an exact age and sex, with the exact background information, and the exact treatment. Estradiol indicated DNA damage in the in vitro micronucleus test, this means that estradiol have the potential of inducing DNA damage. However in vitro tests have their shortcomings, when used to explain if estradiol given in a reasonable amount to a human will express its potential toxicity.

The advantages of using a whole animal body

The greatest problem of in vitro methods is the lack of a body that absorbs the chemical, transforms it to other substances, stores it and then removes it. After estradiol has passed through the body of an animal, only a few percent of it is still available and can perform toxic effects on the cells. A few processes like transformation and storage can partly be added to the in vitro cells, but still we cannot yet mimic the complexity of a whole animal. One simplified solution to this problem is to give much larger amounts of the chemical to the animal than the amount added in vitro, however to get the same complexity as that of absorption and excretion is still a problem. Science does not yet understand all the processes happening in an animal body, how then can we replicate it?

The transformation of the chemical of interest to other chemicals is an important factor as to whether a chemical is toxic or not. In the body estradiol could be transformed to many different metabolites, some of them are very toxic, some are not. It is hard to, in vitro, mimic the metabolism of an animal. However already knowing the metabolites of interest, how they are transformed and in what cells they act, in vitro experiments can give additional information. When a negative result is seen in vivo it could be because of metabolism breaking down the chemical making it less toxic, while in in vitro this effect is smaller thus giving a positive result. A negative result in vivo could also be explained by the great decrease in concentration of the chemical due to absorption, excretion and storage in the body, while this decrease is not present in vitro.

In vitro versus in vivo

In vitro models do have some advantages over in vivo, they can be designed so that we can more accurately study special events, choice of dose, time, organ and animal species can be regulated so that if we know the exact circumstances we can find out the exact results. However if we do not yet know much about the chemical and we want to take a step back and study what happens overall with a whole organism when exposed to a chemical, then we still need an animal experiment. In vitro tests are used in science today, but they are often complemented by animal experiments. The in vitro models are developing and by combining several in vitro tests and some computer models and dolls scientists hope to be able to replace animal experiments sometime in the future. But many are also skeptical to whether this is even possible, because of the great complexity of a living organism. We do not even know all the details of how animals work, how can we then try to copy them? Well, it seems like it is a long way left until all animal experiments can be replaced, but that doesn't mean that it is impossible. Who would for example have predicted the modern mobile phone 30 years ago?

"Science is always moving ahead. The truths of yesterday are todays untruths. And todays truths could be tomorrows untruths."

Thorsten Thorén. Swedish original text to be found in Vetenskapsteori för nybörjare. Translation by the author.

How the micronucleus test works

Some chemicals, like estradiol, have the ability to break lose pieces of chromosomes or whole chromosomes, so that they become lost from the genome. When this happens the lost pieces of DNA are enclosed by a membrane and thus form a small nucleus that has no function but still is visible in microscope. In the micronucleus test the number of such small nuclei is counted before and after exposure of the cells to the test compound. A micronucleus is seen in the bottom picture on the first page.

Additional reading

Carlsson H. 2014. Problematik kring *in vitro* och *in vivo* metoder för toxicitetsstudier: *In vitro* och *in vivo* mikrokärnstest ger olika bedömningar av estradiols DNA-skadande effekt. Independent project in biology at Biology Education Center, Uppsala University

Swedish fund for research without animal experiments / Forska utan djurförsök. http://www.forskautandjurforsok.se

Pictures

Photos by the author and Hernández LG, Benthem J, Johnson E. 2013. A mode-of-action approach for the identification of genotoxic carcinogens. PLOS one, doi 10.1371/journal.phone.0064532.