

Animal welfare and use of silkworm as a model animal

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ABSTRACT: Sacrificing model animals is required for developing effective drugs before being used in human beings. In Japan today, at least 4,210,000 mice and other mammals are sacrificed to a total of 6,140,000 per year for the purpose of medical studies. All the animals treated in Japan, including test animals, are managed under control of "Act on Welfare and Management of Animals". Under the principle of this Act, no person shall kill, injure, or inflict cruelty on animals without due cause. "Animal" addressed in the Act can be defined as a "vertebrate animal". If we can make use of invertebrate animals in testing instead of vertebrate ones, that would be a remarkable solution for the issue of animal welfare. Furthermore, there are numerous advantages of using invertebrate animal models: less space and small equipment are enough for taking care of a large number of animals and thus are cost-effective, they can be easily handled, and many biological processes and genes are conserved between mammals and invertebrates. Today, many invertebrates have been used as animal models, but silkworms have many beneficial traits compared to mammals as well as other insects. In a Genome Pharmaceutical Institute's study, we were able to achieve a lot making use of silkworms as model animals. We would like to suggest that pharmaceutical companies and institutes consider the use of the silkworm as a model animal which is efficacious both for financial value by cost cutting and ethical aspects in animals' welfare.

Keywords: Ethical issue, alternatives, developing drugs, medical studies, 3R, test animals, cost

It is said to be unavoidable to sacrifice model animals for developing effective drugs (1) before being used in human beings. However, use of mammalian animals has not been convenient due to high costs, long breeding times, and large and sophisticated space requirements. Another major limiting factor is ethical issues associated with the use of mammalian models (2). Various alternatives are being developed such as replacing mammals with cultured cells, but the effect of those is still limited (3). Therefore in Japan today, at least 4,210,000 mice and other mammals such as rats, guinea pigs, rabbits, dogs, cats, and monkeys are sacrificed to a total of 6,140,000 per year for the purpose of Medical studies (4).

All the animals treated in Japan, including test animals, are managed under control of "Act on Welfare and Management of Animals" enacted in 1973. The fundamental principle of this law is provided as "In light of the fact that animals are living beings, no person shall kill, injure, or inflict cruelty on animals without due cause, and every person shall treat animals properly by taking into account their natural habits and giving consideration to the symbiosis between humans and animals." (Article 2). This principle indicates that inflicting animals more pain than for inevitable tests is forbidden, even in necessary animal testing (Article 44) (5). It is required to minimize both numbers of test animals and the pain given to them. This principle has been basic and traditional in Western culture, and was already proposed in 1959, as the "3R" principle by W. M. Russell and by R. L. Burch in the UK (6). The three Rs are: "Replacement" – replacing the way of testing without test animals, "Reduction" – reducing the number of them, and "Refinement" – minimizing the quality and the intensity of pain given to animals to the least amount. "Animal" addressed in the principle can be defined as a "vertebrate animal" (7). In Japan, this principle was reflected in amendment of "Act on Welfare and Management of Animals", Article 44 in 2005.

If we can make use of invertebrate animals in testing instead of vertebrate ones, that would be a remarkable solution for the issue of animal welfare. There are numerous advantages of using invertebrate animal models: less space and small equipment are enough for taking care of a large number of animals and thus are

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cost-effective, they can be easily handled, there are less ethical issues surrounding their use, and many biological processes and genes are conserved between mammals and invertebrates. Many invertebrates have been used as animal models: fruit fly *Drosophila melanogaster* (8,9), grasshopper *Romalea microptera* (10), wax moth larva *Galleria mellonella* (11), honey bee *Apis mellifera* (12), and silkworm larva *Bombyx mori* (13). Silkworms have many beneficial traits compared to mammals as well as other insects. Silkworms are not only easy for injection experiments but also they can be injected either through the intrahemolymph route that corresponds to intravenous in humans or the intramidgut route that corresponds to oral in humans. Figure 1 shows the easy ways of injecting silkworms through these routes. Unlike *D. melanogaster*, the silkworm has a large enough body size to carry out experiments for accurate dose administration and organ isolation. While injection into *Drosophila* requires special techniques and even though female flies are larger, they however have a hard outer surface which makes them difficult for injection. Unlike

R. microptera and *G. mellonella*, the silkworm has an established method of breeding as it has been used for silk for over five thousand years. Unlike honey-bees, silkworms do not bite and even the adult moth cannot fly. The larvae do not have any sharp hair or horns that sting, so they are not harmful and do not require special techniques and caution for use. Moreover, the locomotion of silkworm larvae is slow which ensures that it cannot escape away from the laboratory setting easily which minimizes the risk of biological hazards. They can be fed an artificial diet that is easily available and can be bred all year round so that there is no shortage of larvae for experiments. The established rearing method allows the same kind of larval breeds which is very important for reproducible results. Larvae molt four times, it is easy to separate each instar larvae and they are stable having little individual genetic differences that give uniformity in research results. The whole genome is now known which allows for genetic manipulations and there are already many ongoing research projects that utilize transgenic and genetically modified silkworms.

Different models of the silkworm have already been established: bacterial infection model (13,14), baculoviral infection model (15), model to test innate immunity (16-18), diabetic model (19), bacterial virulence model (20-22), model to test pharmacokinetics (23), and model to test toxicity and metabolism (24). Most of these research projects have proven the correlation between results in mammals and silkworms. Table 1 distinguishes various features of the silkworm with other insects.

In the Genome Pharmaceutical Institute's study, we were able to achieve a lot making use of silkworms as model animals. Not only did we discover new effective chemicals including a new antibiotic "Kaikosin", but also we reduced the number of mice (small mammals in standards of test animals' size) and that helps towards making a solution for problems in animals' welfare, and cutting costs of testing. We are using silkworms instead of mice in the initial screening and testing in

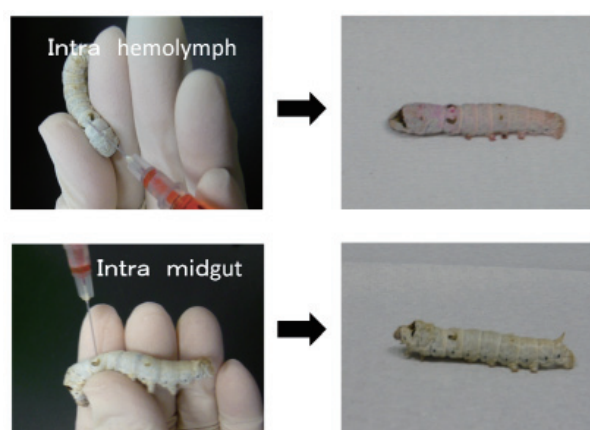


Figure 1. Injection into hemolymph and midgut. When injection angle is shallow, red ink is injected into blood (Upper). On the other hand, when injection angle is deep, it is injected into midgut and silkworm is not stained (Lower).

Table 1. Comparison of silkworm with other insects

Items	Silkworm	Drosophila	Honey bee	Waxmoth	Grass hopper
Size	40-60 mm	1-3 mm	15-17 mm	30-40 mm	60-80 mm
Breeding method	Well established (> 5000 years)	Well established	Well established	Established	Established
Locomotion	Larva: slow, Adult: cannot fly	Flies	Flies	Larva: faster than silkworm, Adult: flies	Jumps, flies
Special handling technique	Not required	Required	Required	Not required	Required
Chance of biohazard	Less	Higher	Higher	Higher than silkworm, less than others	Higher
Injection technique	Easier, anyone can learn within couple of hours	Difficult, requires skilled personnel	--	Easier	--
Isolation of organs	Easier	Difficult ,not always possible	Easier	Easier	Easier
Route of administration/ Accuracy of administrated dosage	Oral, injection to dorsal surface: intrahemolymph, intramidgut/ accurate in case of injection	Oral, injection to dorsal surface, not accurate	--	Oral, topical, injection to ventral surface/ accurate in case of injection	--
Diseases models	Many	Many	--	Few	Few

Table 2. Experimental models used in screening antimicrobial agents from 10,000 chemical compounds and 15,000 natural compounds

Experimental models	10,000 chemical compounds	15,000 natural compounds
Our testing method	100 mice + 30,000 silkworms	150 mice + 45,000 silkworms
General method	30,000 mice	45,000 mice
'Reduction' effectiveness of mice	30,000 → 100 mice	45,000 → 150 mice

our company which is said to be a 'Replacement'. We used silkworms for screening chemical compounds as well as natural compounds. In screening for a curative effect with 10,000 chemical compounds' samples and 15,000 samples of products from soil bacteria (natural compounds) with antimicrobial activity, the number of mice used as test animals in our testing method is obviously less than that in the general method which here refers to tests in a mouse model directly after *in-vitro* analysis. We used silkworms after *in-vitro* analysis and before testing in the mouse model, and thus we could decrease the number of mice used for the tests. This decrement in the number of test mice is the 'Reduction' effectiveness (Table 2).

As shown in Table 2, we have gained a remarkable result in reduction of the number of mice in testing, by replacing them with silkworms in the initial testing. Having these results, we have been suggesting to pharmaceutical companies and institutes to consider the use of our silkworm related technologies, which is efficacious, with both a financial value by cost cutting and ethical aspects in animals' welfare. If we can replace 10% of tests run using mice as test animals in Japan, that means at least 400,000 mice can be saved from testing per year.

We should be aware of the fact that in Europe, more strict regulation, Cosmetics Directive 76/768/EEC was enacted in 2003. Dr. Tsutomu Kurosawa, the president of the society refers to the directive as "One of the epochs of our activities was the 7th amendment of EU cosmetics Directives in 2003. This directive ordered the total abundance of animal experimentation for cosmetic development and trade." (25). Based on this regulation, animal testing is gradually banned for developing materials and products for cosmetics, and products, subject to regulation of this Directive are already banned for sales in the EU. Some of the whole tests are still run, since a substitute way of testing without use of animals cannot be found, and it will take some more years for total abolishment. However, it won't be long before the day cosmetics produced using animal testing will be banned from sales in the EU. This is the global trend, and in the near future, animal usage alternatives or use of creatures, not controlled by the concept of animal welfare will be more valuable. With these issues, the silkworm is thus an ideal living creature for testing and can be counted on as a reliable test animal having great potential without animal welfare regulations.

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