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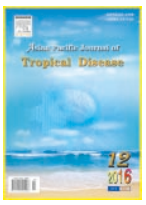

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Elementary of animal model for percutaneous and ocular penetration

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ABSTRACT

Models of animal are the most appropriate method for assessments of human *in-vivo* percutaneous and ocular penetrations. Monkey and rodents are used for the same. There are several nuts and bolts of each one, so it is necessary to study each one separately. Monkey, porcine and guinea pig penetration are correlated with that of human skin. The skin of rodents, lupus, pigs, *etc.* has more penetration properties than human skin. Rabbit, goat and sheep eye are mostly used for ocular penetration. The researcher also used hen's egg chorioallantoic membrane test for ocular irritation study. The other animals' cornea, *cul-de-sac*, eyeballs and prepared corneal epithelial models are very less in practice. Web-based alternative non-animal models are also available instead of animal models too. This article describes characteristics of monkeys, pigs, rats, rabbits, guinea pigs and hairless rodents, HuSki model, Cellophane® membrane, egg membrane, gelatin membrane, animal models for ophthalmic delivery, hen's egg chorioallantoic membrane test, prepared corneal epithelial models and web-based alternative non-animal database.

1. Introduction

A best appropriate method to determine the percutaneous and ocular drug flux of humans is *in-vivo* studies. However, it is hard to carry out *in-vivo* human studies due to ethical issues, patients' consent, *etc.*[1]. But *in-vitro* human skin or ocular penetration study is not stopped by these issues and Helsinki's Declaration[2]. There are differences in physiological and metabolic conditions of animals and human. That is why animal models are in limited in practice. Animal models are more practical because they are easily available, and fewer issues of the ethical committee, less differences between subjects, and large numbers of data could be evaluated related to ocular, percutaneous penetration, toxicokinetic and toxicodynamic studies[3]. Skin of rodents like rat or mouse is thinner than human skin. It has different lipid content, more enhancement ratio, and chemical modification than human skin. To get most relative data to the human penetration, animal models should have physiological, biochemical and anatomical equality to humans. Animals close to humans in such criteria are good models, but it is not the absolute necessity for an animal to be genetically similar to humans. The study indicated that an animal genetically close to human could have organ characteristics similar to humans[4]. Several basic criteria are considered to judge whether an animal is most relevant or not.

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2. Monkey

The monkey is the most relevant animal model for permeation because it is phylogenetically most close to humans. Moreover, hair density of monkey skin is also similar to those of humans too. Its skin is similar to human skin and areas of the inner arm, legs and trunk are also hairless like human skin. Its regional variation in ocular and percutaneous absorption is like human. That is why its anatomical portion could be used in the study. Moreover, it is large enough for serial blood sampling. Due to the cost, handling and availability problems the use of monkeys in *in-vivo* studies is limited so far. However, there are differences in the skin anatomy of the monkeys and humans. Monkey is covered with a thick coat of pelage and without hairs. Its epidermis has somewhat under sculpture. There are plenty of apocrine glands at the root of hairs. It has fewer numbers of sebaceous glands and it strictly opens to the skin surface also. There were several studies on monkey skin that found that several chemical entities had almost equal permeability in monkey skin and human skin. That is why percutaneous and ocular absorption across monkeys often, but not always, resembles human[5].

3. Pigs/porcine

Other than the monkey, the most appropriate animal model for human skin penetration is pig for both *in-vivo* and *ex-vivo* studies. Porcine skin is easily available from a slaughter house as well. Moreover, the pig is also large enough for samplings of pharmacokinetic and pharmacodynamics studies for a longer period

of time. It is not difficult to handle in standard animal house. There are several similarities between porcine and human skin anatomically (Table 1) and physiologically (Table 2). Its skin is made up of hair coat and thick epidermis, under the sculpture, a dermis with the papillary body and large numbers of elastic tissues.

Table 1
Thickness of skin layers and cornea of different species[6].

Species	SC (μm)	Epidermis (μm)	Whole skin (mm)	Number of hair follicles/cm^2[7]	Cornea[8] (mm)
Human arm/eye	17.00 ± 1.00	40.00 ± 4.00	2.30 ± 0.50	60 ± 5	0.95 ± 0.05
Monkeys	20.50 ± 2.30	26.90 ± 3.10	5.00 ± 1.00	71 ± 8	N/A
Porcine	12.30 ± 0.75	51.90 ± 1.51	3.40 ± 0.30	20 ± 3	N/A
Rats	14.00 ± 1.15	22.20 ± 2.35	1.10 ± 0.27	299 ± 29	N/A
Rabbits	6.60 ± 0.41	11.10 ± 1.10	1.95 ± 0.25	8000 ± 20	0.41 ± 0.02
Guinea pigs	25.80 ± 0.52	66.10 ± 3.10	3.51 ± 0.21	12 ± 2	N/A
Goat	N/A	N/A	N/A	N/A	0.71 ± 0.03
Sheep	N/A	N/A	N/A	N/A	0.84 ± 0.01
Buffalo	N/A	N/A	N/A	N/A	1.14 ± 0.05

Values are mean ± SE; SC: Stratum corneum; N/A: Not applicable.

Table 2
Blood flow measurements[9].

Species	Buttocks (mL/min/100 g)	Pinnae (mL/min/100 g)	Humeroscapular joint (mL/min/100 g)	Thoracolumbar junction (mL/min/100 g)	Ventral abdomen (mL/min/100 g)
Monkeys	3.12 ± 0.58	20.93 ± 5.37	8.49 ± 3.28	2.40 ± 0.82	3.58 ± 0.41
Porcine	3.08 ± 0.48	11.70 ± 3.02	6.75 ± 2.09	2.97 ± 0.56	10.68 ± 2.14
Rats	4.20 ± 1.05	9.13 ± 4.97	6.22 ± 1.47	9.56 ± 2.17	11.35 ± 5.53
Rabbits	3.55 ± 0.93	8.38 ± 1.53	5.38 ± 1.06	5.46 ± 0.94	17.34 ± 6.31

Values are mean ± SE.

Tissue turnover time, structure, numbers of bundles, the thickness of collagen fibers, monoclonal immunoreactivity, polyclonal antibodies, filament density, areas of cell overlapping number, size, distribution, the dermal blood vessels communications, enzyme patterns, keratinous proteins, glycosphingolipids and ceramides characteristics of the porcine and human epidermis are similar. Rich vascularization is found in human, but that is poor in pigs. The human has most of the eccrine type sweat glands, whereas pig has most of the apocrine type glands. Several studies proved that there would be the strong positive monotonic correlation between the permeation of the human and porcine skin. The permeability of chemical entities through pig skin and human skin could be better correlating. The ranking could be very similar, but absolute permeability could be different[10].

4. Rodents

Rodents like rat and mouse are readily available, small, easy to handle, cheap and easy for sampling data. That is why they are most commonly used in permeation studies as well as regulatory toxicity and sensitivity studies. Skin of rat and mouse is thinner than human stratum corneum and have different lipid composition. Thus, it is more susceptible for enhancement and chemical modification than human skin, so it is a relatively poor model[11]. Among rodents, rat skin has more anatomical similarities to the human skin. Therefore, rat skin is frequently used for permeation kinetic studies. However, rat skin has higher appendage number and fewer corneocyte surfaces than human skin. There are more than one million papers published using the rat as the model for *in-vivo* or *ex-vivo* studies. Factors of difference (FOD) between skins of rat and human is also required in the comparable range[12].

To overcome the problem of FOD several research groups were suggested a parallelogram, to find dermal penetration for human skin by using *in-vivo* and *in-vitro* rat data and human *in-vitro* data by the following equation:

$$\%\ \text{Human penetration} = \frac{[\textit{In-vivo}\ \%\ \text{penetration in rat}] \times [\textit{In-vitro}\ \text{rate of penetration in human}]}{\textit{In-vitro}\ \text{rate of penetration in rat}} \quad (1)$$

There could be very good correlations found between estimated and measured values of human *in-vivo* dermal penetration. The parallelogram method is also used for the other than rat animal models[13].

5. Rabbits

Like rodents, rabbit skin is also more permeable than human skin. There is no consistent difference in percutaneous absorption between rabbit skin and human skin. Rabbit ear skin had hair follicle (80 ± 2)/cm^2 and shows comparable permeability in some molecules like celecoxib, buspirone and ibuprofen. The rabbit ear skin is a competent model to study iontophoretic transport of drugs. Its *in-vitro* electro-osmotic and electro-repulsive transport are almost similar to those of human skin[14]. Rabbit ear skin and pig ear skin has the thickness of stratum corneum similar to human skin. The lipid compositions are different. Pig ear has a higher content of nonpolar lipids. Viable epidermis of rabbit ear is much thinner. Hair follicle density is also higher than pigs and humans. Rabbit ear has higher lipophilicity of its stratum corneum than that of human skin.

6. Guinea pigs

Unlike the other rodents, guinea pig skin is not more penetrative than human skin. There is an excellent correlation existence between guinea pig skin and human skin permeability ($0.3 < \text{FOD} < 3.0$), but no correlations between leg time of both of them ($\text{FOD} > 3$). Higher hair density in guinea pigs may contribute to the high permeability of guinea pig skin for hydrophilic drugs like salicylic acid, chloramphenicol, paraquat dichloride and NaCl, *etc.*[15].

7. Hairless rodents

Rodents have one of the disadvantage which is extremely high hair follicles density. Therefore, it required to remove hair removal before experimental studies which can affect percutaneous absorption of entities. To overcome these issues, hairless rodents have been used[4].

7.1. Hairless rats

In earlier studies there were only hairless rat models used for *in-vivo* studies. There could be relatively larger surface depots but much lower local accumulation for hydrophilic entities like salicylamide, which is so advisable and used for lipophilic entities only[16].

7.2. Hairless mice

Fat content of human skin changes from area to area thus prediction of the permeation data is difficult to study, while hairless mouse *e.g.* stratum corneum of rhino mouse skin has constant fat content so that this issue could be subsided. Stratum corneum fat composition of mouse skin is almost the same to that of human skin. The whole body of hairless rhino mouse skin could be available for *in-vivo* studies too. It is used for assessments of permeation for human skin with defined protocols[17,18]. Rhino mouse skin is less thicker than that of a human. It is more susceptible to chemical

perturbations than human skin, so FOD > 3. For *in-vitro* studies, hairless mouse skin needs to be hydrated thoroughly before the experiment. The relative effect of each enhancer formulation on the two skins was not consistent and therefore the hairless mouse model should not be used to predict the effects of penetration enhancers in human skin[19,20].

7.3. Hairless guinea pigs (HGPs)

Unlike hairy guinea pig, skin of HGP has some structural similarities with human skin. The HGP epidermis is as thick as human skin and has 5–10 layers which are similar to human epidermis. Thickness and the number of blood vessels are similar. They could be an excellent correlation between HGP and human skin for permeability and lag-time. HGP skin is slightly more permeable but close to that of human, so HGP skin is a good alternate for human skin (0.3 < FOD < 3)[21].

8. Innovative human skin grafted onto nude mice model

For comparison of skin absorption, retention and permeation among rat *in-vivo*, human and rat *in-vitro* studies, an innovative human skin grafted onto nude mice model is also utilized. It closely predicts the human skin penetration. Moreover, evaluation over extended periods of time is also feasible[22].

9. Cellophane® membrane

Cellophane® membrane No. 300 is mostly preferred. Cellophane® membrane is a brand of cellulose membrane. It is 100% cellulose, free from fat content. That is why it is used for the purpose to evaluate permeability of formulations when penetration enhancers are present in it. Its thickness is 40 μm but it is impermeable, so it could be dipped in water for 24 h and then in 1% zinc chloride solution for one day or heated with 0.1 mol/L NaOH for half an hour. The pore diameter of it after the treatment is 80 μm and thickness is 30 μm[23].

10. Egg membrane

Egg membrane could be prepared by treating egg with 0.1 mol/L HCl for 3 days. The HCl reacts with calcium and aids to remove the outer shell of the egg membrane. The reaction was observed by a development of air bubbles. The separated outer membrane could be washed with running water. This could be used for diffusion studies, but high variation in the diffusion rate of the drug through egg membrane was observed when compared with human skin, so it is not reliable to take for permeability studies[24].

11. Gelatin membrane

Gelatin membrane is made up of porcine skin whose pore size after heating with 0.1 mol/L NaOH is 80–90 μm and thickness is 30–40 μm. Gelatin membrane is used as a model for permeability of the human skin[25].

12. Animal model for ophthalmic delivery

Animal experimentations are important in the research and development of ocular delivery systems. Therefore, live animals have been utilized to assess the pharmacological effects. The rabbit is the most widely used animal model while pigs, monkeys, dogs, and cats are also used. Mice and rats are less frequently used in studies due to their small eye size. There were permanent eye injuries caused by the cosmetic dye sold in the 1930s, and the Food and Drug Administration of the United States developed the rabbit *in-vivo* Draize test for assessment of acute ocular toxicity[26]. Draize test is an international standard bioassay in which New Zealand white rabbits are used because they are relatively cheap, obtainable and have large eyes. Rabbit cornea has been preferred in the majority of the permeation studies which has now been restricted by most of Animal Ethical Committees across the globe[8]. Rabbit cornea and eye model used to assess increase in corneal thickness and opacity, ocular sensitivity, the possible limitation for solids and corrosion, active transport studies and permeability. Bovine cornea could be used for ocular sensitivity and corrosion testing. Buffalo cornea could be also used for ocular toxicity studies. The isolated chicken eye could be also used to study an increase in corneal thickness, permeability, opacity, ocular sensitivity and corrosion. It has possible limitation for solids[27]. Eyes of goat or sheep are frequently used as animal models for diseases of humans.

12.1. Hen's egg chorioallantoic membrane

Ocular irritation of ophthalmic formulations could be checked by hen's egg chorioallantoic membrane test. It is a rapid, sensitive and inexpensive test. In the test incubated eggs are used. This *ex-vivo* test does not conflict with the ethical and legal issues. The prepared chorioallantoic membrane is a complete tissue and is easy to study. It reacts to injury with inflammatory conditions too[28].

12.2. Prepared corneal epithelial models

Epiocular™ and Skinethic™ are used for ocular sensitivity and corrosions, and Clonetics™ is used for ocular irritation and transepithelial permeability studies. Immortalized Statens Seruminstitut rabbit corneal cells were used for corneal drug metabolism and transport. Immortalized conjunctival cell line is used for ocular surface defence mechanism[27].

13. Web-based alternative non-animal models

At present animal testing method is used in the topical and ocular dosage formulations to confirm product safety, but it is the obsolete study. The alternative methods to replace the animal studies are in development. Research data concerning to test substances are not feasible for developing novel alternative tests and safety information on excipients has neither been collected in a database nor shared among researchers. Therefore, it is difficult to build and share a safety information on toxicological mechanisms and pathways collected through *in-vivo*, *in-vitro* and *in-silico* methods. There is development of the Consortium of Alternative Methods for Safety Evaluation of Cosmetics database to overcome these issues by researchers of Korea[29].

14. *In-vitro* species comparison and *in-vitro/in-vivo* correlation

Unlike to *in-vivo* animal study, *in-vitro* animal models are easily available, easier to perform and could be provided results in a shorter span of time.

15. Conclusion

Each animal model has its merits and demerits. To develop correlation between animal, human skin and ocular membrane penetration, it is required to know the properties of each animal model and its regulatory requirements. Porcine, hairless guinea pigs and rabbit, goat or sheep are the most appropriate animal models of human skin and ocular penetration among available laboratory animals. Prepared corneal epithelial models are in practice and web-based alternative non-animal models will be also in practice soon.

Conflict of interest statement

We declare that we have no conflict of interest.

Disclaimer

Any opposite opinions, findings, conclusions or recommendations expressed in this material are those of the corresponding author only.

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