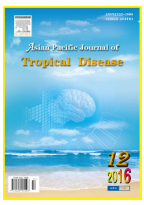
HOSTED BY

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Review article doi: 10.1016/S2222-1808(16)61174-X

Elementary of animal model for percutaneous and ocular penetration

Kalpesh Chhotalal Ashara*, Ketan Vinodlal Shah

School of Pharmacy, RK University, Rajkot-Bhavnagar Highway, Tramba, Rajkot 360020, Gujarat, India

ARTICLE INFO

Article history:
Received 15 Aug 2016
Received in revised form 24 Aug, 2nd revised form 7 Sep 2016
Accepted 15 Oct 2016
Available online 31 Oct 2016

Keywords:
Animal models
Skin penetration
Ocular penetration
Factors of difference

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.

*Corresponding author: Kalpesh Chhotalal Ashara, School of Pharmacy, RK University, Rajkot-Bhavnagar highway, Tramba, Rajkot 360020, Gujarat, India.
Tel: +91 9586407672
E-mails: kalpeshshr5@gmail.com; kalpesh.ashara@rku.ac.in
The journal implements double-blind peer review practiced by specially invited international editorial board members.

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