The use of minipigs in dermal and wound healing research

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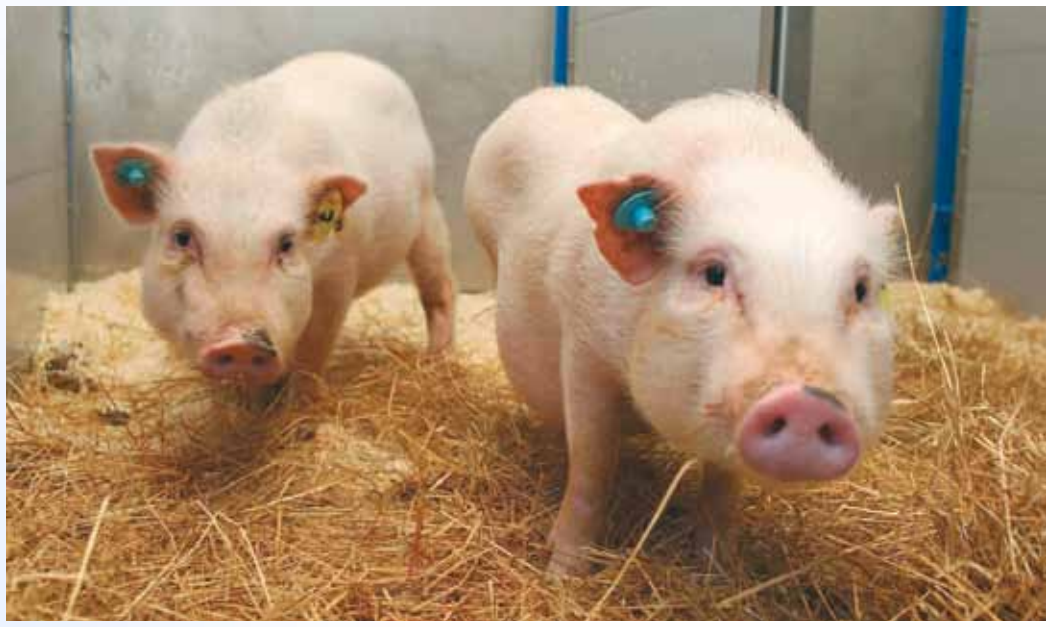
Introduction

In general animals are used in safety and efficacy research as models to predict effects in humans. Therefore, the challenge is always to identify the most relevant animal species, i.e. the species that shares the relevant features with the target species, being humans. Regarding dermal research, the pig is an ideal model due to the high degree of similarity of the skin of this species to human skin, and in this regard no other animal species exhibits such a high degree of resemblance.

As in humans the skin of the pig is more firmly attached to the underlying structures in comparison with for instance dogs and rabbits, where the skin is loose. Also, the hair coat of pigs is sparse and the skin surface is carved by fine intersecting lines, which is also the case in humans. Microscopically, both humans and pigs show resemblance in terms of skin thickness, the presence of antigen-presenting Langerhans cells and rete ridge formation in the dermis. Similarities are also seen in transdermal absorption of a variety of different drugs, although this depends on a number of factors including skin site, temperature, humidity, occlusion and skin condition.

Due to the smaller size and thereby a reduced test substance requirement, the use of minipig species is advantageous. The Göttingen minipig is especially useful for the above mentioned studies, as the skin is non-pigmented which facilitates dermal- and wound observations.

Picture 1. Göttingen minipigs



Dermal toxicity studies

The similarity between the skin of humans and pigs makes the pig an ideal model for use in non-clinical dermal studies. In drug development the most appropriate animal species should always be used for non-clinical safety testing, and for pharmaceutical products intended for dermal application it is therefore difficult to justify not to use the pig for these studies.

As a standard, the test item is dosed on an area corresponding to approx. 10 % of the total body surface area. Normally the skin on the back is used, as this area is protected from self-inflicted injuries and oral ingestion of test material and is furthermore easy to cover with secondary protecting bandage materials, and as the area is not normally subjected to external contamination with passed urine and faeces.

During dosing, the treated area is usually covered by a gauze dressing, held by a net-like bandage covering the thorax and abdomen. It is of great importance to allow a daily treatment-free period, as the risk of dermal irritation and skin infections otherwise increases due to the moist, warm, semi-occlusive environment. We routinely use a daily dosing period of six hours, although other durations are possible. At termination of the dosing period, any remaining test substance is removed and washed off.

Picture 2. Minipig dermally dosed

According to guideline requirements for toxicity studies, other assessments like ophthalmoscopy, electrocardiography, blood sampling for clinical pathology and toxicokinetics and necropsy procedures should be included in the study design. These procedures, performed routinely at our laboratory and are easily performed under the dosing conditions described above. For each parameter measured an extensive historical control data base exists.

Wound healing studies

Also in terms of wound healing, the pig constitutes a much better model than all other animal species. Again, the similarity to humans regarding the skin is important. In addition, the wound healing process consists of the same phases as in humans (inflammation, contraction, proliferation, re-epithelialisation and re-modelling) and for both humans and pigs, where the skin is firmly attached to the underlying structures, wound contraction occurs quite differently as compared with loose skinned animal species like rats, rabbits, dogs and non-human primates.

For the same reasons as stated above, the wounds are established on the back of the animal. Furthermore, the area is supported by the vertebrae and the ribs, making skin tension limited, thereby reducing interference of this factor with wound contraction. Depending on the size of the animals at the start of a study, up to eight wounds can be made on each animal.

Full-thickness wounds involve removal of epidermis, dermis and subcutis, whereas only part of the epidermis is removed in split-thickness wounds. The full-thickness wounds, healing by granulation and re-epithelialisation with scar tissue formation, are made in circular shape with a diameter up to 20 mm. The circular shape provides the most uniform wound contraction. The split-thickness wounds are formed square and the maximum size and depth of these wounds are 40x40x1.2 mm. These wounds heal by re-epithelialisation with no scarring.

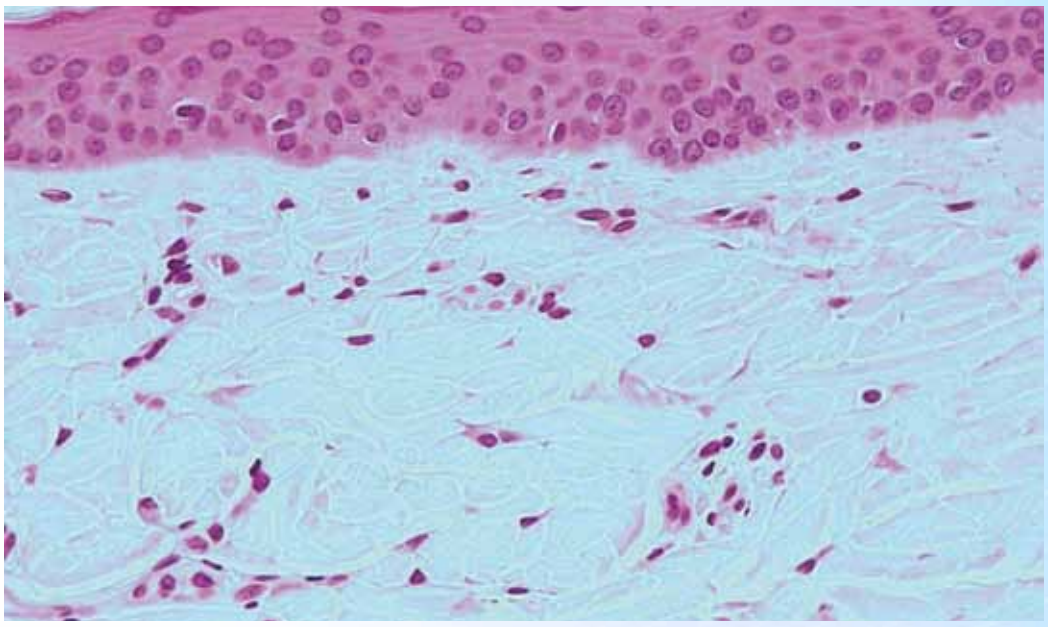
Following wounding, the wounds are treated with test item (gels, ointments or dressings) and further covered by secondary dressings. Dosing can be performed on a daily basis, depending on the intended human application scheme. During the wound healing phase the wounds are evaluated and subjected to detailed macroscopic evaluation, including planimetric recordings. Terminal observations include microscopic evaluation, with special stainings for collagen deposition and angiogenesis. Also, concentration of newly formed collagen within the wound tissue can be indirectly measured by analysis of 4-hydroxyproline concentration.

Local tolerance studies

During a drug development process it is a requirement to test for local tolerance. In some instances this element can be integrated into single or repeat dose studies. However, if more detailed observations need to be made, including sampling of tissue at different time points in relation to dosing, separate studies with local tolerance as the major objective need to be performed.

For screening purposes of dermal compounds, it is possible to test several formulations using the same animal, as based on the size of the animal. Thereby, inter-animal variation can be minimised. Test sites are usually indicated by tattooing and each site is treated and bandaged as described for dermal toxicity studies.

Picture 3: Minipig skin, epidermis and upper part of dermis



Abraded skin model

Under normal conditions, the skin constitutes a relatively profound barrier to the external surroundings. However, in relation to some human skin diseases like for instance psoriasis, superficial abrasions and sores develop, potentially increasing systemic absorption of substances intended for topical skin use. This issue is not covered when including healthy animals with intact skin in regulatory toxicity testing of dermal products. For such studies, development of a model for repeated administrations on abraded skin has therefore become relevant.

The major problem with abrasions is how to standardise the process? There are many variables, e.g. how to abrade (scarifying, tape-stripping), when to abrade (pre study, during treatment), how often and whether to attempt abrasion in animals that are showing clear dermal reactions to treatment. We have therefore developed a model of compromised or broken skin that is properly controlled and reproducible, based on a wound healing model during the initial part of a study. For legislative and animal welfare reasons it is not possible repeatedly to establish new wounds on the animals upon complete wound closure, which would be necessary to evaluate the effects of test substances applied on abraded skin over longer periods. Therefore, the initial wound healing phase is combined with a phase of dermal or subcutaneous dosing, starting at the time of complete re-epithelialisation of the wounds. This meets requirements for regulatory non-clinical studies where dosing is to be performed during longer periods. Subcutaneous dosing ensures maximal systemic exposure of the test compound, taking increased systemic absorption through wounds and abrasions into account, although not being the clinical relevant route of dosing. Alternatively, dermal dosing can be used as the secondary route of dosing in some instances. The model has been accepted by the regulatory authorities.

Juvenile studies

During the most recent years, increased regulatory requirements to new chemical entities with paediatric indications have been implemented. Per default, a Paediatric Investigational Plan, including preclinical juvenile studies, should be addressed, whenever relevant. A need for developing juvenile animal models has subsequently been necessary.

The Göttingen minipig constitutes an ideal juvenile model due to the relatively long developmental period following birth, enabling investigations at variable paediatric life phases. In addition, a litter size of 5-6 offspring in average, reduce the number of sows to be included in a study. In order to reduce the genetic relationship between individuals included in the same dose group and to eliminate the risk for contamination between groups, we have developed a method for cross-fostering of the animals.

Picture 4: Minipig sow with litter

For test compounds intended for oral, subcutaneous, intramuscular of intravenous administration, dosing can be commenced from day 7 of age. However, for dermal compounds it is not practically feasible to start dosing before after weaning, preferably at an age about 5-6 weeks. At this age the animals can be housed individually during dosing, eliminating the risk of bandage destruction and oral ingestion of test item by litter mates. In order to cover earlier phases of life, dosing can eventuall

Conclusion

In conclusion, the pig and in particular the Göttingen minipig, constitutes a valid and practically feasible model for evaluation of test compounds intended for dermal administration or for administration topically on split- or full-thickness wounds. Also, in terms of juvenile testing, the minipig is superior in comparison with other animal species.

For regulatory toxicity studies, for screening purposes and for efficacy testing the minipig should therefore always be considered as a relevant test species in relation to nonclinical development of products for dermal use or within wound care. The minipig is fully accepted globally by regulatory authorities and considerable historical control data for the Göttingen minipig has now been generated over at least the last two decades.