Age-related changes in hematopoietic activity in the bone marrow, spleen and liver in neonatal, juvenile and young/adult Göttingen minipigs

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Introduction

There is a rising interest in the normal histological appearance and the age-related microscopic changes in juvenile Göttingen minipigs, due to the increased use of young pigs in preclinical toxicological studies with pediatric drug candidates. In mammalian development, the hematopoietic tissue sequentially occupies the embryonic yolk sac, fetal liver, spleen and bone marrow. In large animal species, during maturation from neonate to adult, the hematopoietic tissue tends to decrease and the spleen and liver generally no longer harbor any hematopoietic tissue in healthy individuals from young-adult onwards. In adults, the hematopoietic tissue generally persists in the axial bones (sternum, vertebrae and ribs) and epiphyses of long bones. The relative proportion of hematopoietic to adipose tissue in marrow is highly variable and decreases with age. Bone marrow represents 2-5% of the body weight in adult animals (ref.1).

Results

Histopathology

Bone marrow (Fig. 1 to 10) – High cellularity was observed in the sternum and femur at PND14 and PND35, with only a few scattered small adipocytes present at PND35. At PND63, the amount of adipose tissue increased with age. In the femur, adipocyte infiltration appeared first in the epiphysis. Serum alophy of the bone marrow was not seen in animals up to PND90 and was sporadically observed in the femur in 5-6 months-old pigs, generally males. Spleen (Fig. 7 to 9) – The red pulp exhibited high cellularity due to sustained diffuse hematopoietic activity at PND7 and PND14. The hematopoietic activity decreased with age and was only minimally observed at PND35 and no longer observed at PND63. Macrophages engulfing basophilic, variably-sized cell debris (so called "Tingible-body macrophages") were observed throughout the hematopoietic tissue in the spleen (PND7 and PND14) and the sternum (PND14). Small scattered pigment deposits consistent with hemosiderin were observed at each time point. Liver (Fig. 11 to 14) – Several, variably-sized hematopoietic foci were observed throughout the liver parenchyma at PND7. The hematopoietic activity was less prominent at PND14, and only a few scattered foci were observed at PND35. Generally, no hematopoietic foci were observed in the liver at PND63 in 5-6 months-old pigs. Pigment deposits consistent with hemosiderin were observed in sinusoidal Kupffer cells. Additionally, prominent diffuse hepatocellular vacuolation consistent with glycogen accumulation was observed at PND7 and PND14. This was attributed to the unfasted state prior to necropsy.

Organ Weights

The body weight and the absolute weight of the brain, spleen and liver increased with age. In 5-6 month-old pigs of both sexes, considerable individual variation was noted for all four weight values. The mean brain:body weight ratio decreased with age (data not shown).

In the spleen (Fig.15 and 16), the individual variation in absolute and relative was prominent in both sexes. Although no definite age-related trend could be identified, it was noted that the mean spleen absolute weight slightly increased from PND7 to PND63. The mean spleen/body weight ratio slightly decreased from PND14 to PND63, and increased from PND63 onwards. The mean liver absolute weight (Fig.17 and 18) only marginally increased from PND7 to PND35, the increase was more pronounced at later time-points. The mean liver/body weight ratio decreased from PND7 to PND35, then increased through PND63 and finally decreased again by 5-6 months of age.

Histopathology (reticulocyte count)

The absolute reticulocyte count (Fig.19) decreased with age and correlated with the age-related decreases in hematopoietic activity in the liver and spleen as well as the decreased cellularity of the bone marrow in the sternum and femur.

Conclusion

The microscopic appearance of the hematopoietic tissue varies depending on the anatomic location and the age of animals. This must be taken into account while identifying and interpreting potential toxic effects in the hematopoietic system. After birth, the liver and spleen progressively lose hematopoietic activity, and in healthy adults only the bone marrow maintains this function).

In the present study, prominent hematopoietic activity was observed in the liver and spleen in 7- and 14-days-old pigs, in addition to the high cellularity of the bone marrow in the sternum and femur. High cellularity of the bone marrow was still observed in 35- and 66-days-old pigs, whereas only minimal or no hematopoietic activity was seen in the liver and spleen at these ages. Similar results have been reported by Kuper et al. (2016), who noted that the bone marrow in the sternum after birth continues to be a hematopoietic site and shows only minimal adipose tissue replacement from day 60 onwards. In the spleen, hematopoiesis is present at birth, which slowly declines and is absent at day 60 and in older pigs. Tingible-body macrophages were seen in the hematopoietic tissue of the bone marrow and spleen in 7- and 14-days-old pigs. This represents a normal feature which is considered to correlate with the physiological decline of the hematopoietic activity noted post-natally.

The prominent individual variation in spleen weight noted in the present study may have been related to spontaneous variation in red pulp exsanguination.

Experimental Design

We examined the hematopoietic tissue in the bone marrow (sternum and femur), spleen and liver in neonatal, juvenile and young/adult Göttingen minipigs. Animals included:

- Post-Natal Day (PND): 7-2 females
- PND14: 4 males + 2 females
- PND35: 17 males + 23 females
- PND63: 11 males + 1 females
- 5-6 month-old: 25 males + 25 females

Pigs were untreated or were given water or cyclodextrin (HP-B-CD) by daily oral administration from PND1. In addition, we assessed absolute and relative spleen and liver weights. Correlating changes in reticulocyte count were also discussed. Since there were no differences in hematology and histology between the untreated/water dosed and HP-B-CD for dosed minipigs up to 6 months, data were combined.

Bibliographic References
