IMMUNOTOXICOLOGY EVALUATION IN THE JUVENILE GÖTTINGEN MINIPIG

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INTRODUCTION

Safety evaluation of new pediatric medicines is performed by the conduct of toxicology studies using juvenile animals. The Göttingen minipig is now considered as a useful alternative non-rodent species for safety testing of pharmaceuticals. Human parallels in many features of its anatomy, physiology and biochemistry make the minipig a good model for man. For use in juvenile toxicology studies, the development of main organs or systems of the minipig still requires further characterisation, the immune system being one of the main areas to be explored. Although the immune system of the adult pig has been studied, there is a need to better understand the immune system organization and response in minipig to better evaluate the toxicological effect of new pharmaceuticals in development in this species.

MATERIALS AND METHODS

Thirty-six piglets, 18 males and 18 females, were born in our animal facilities from Göttingen sows provided by Ellegaard (Denmark) and were raised with their respective mother under optimal housing conditions until weaning, i.e. 4 weeks after birth. During the 4-week preweaning phase, 9 male and 9 female piglets were treated with Cyclosporin A (Neoral® Novartis) at the dose level of 10 mg/kg/day by oral route (gavage) from PN2 (Post Nalatal Day 2) to PN28 (figure 1). Another group of 9 males and 9 females was treated with water as negative control under the same experimental conditions as for the group treated with Cyclosporin A (CsA).

After weaning, the animals were group-housed. The study design was approved by the Animal Ethical Committee and conducted in compliance with the European Animal Welfare Guidelines in an AAALAC accredited Test Facility.

RESULTS

3.1 Immunophenotyping data – Table 2 and Figure 2

- A trend towards higher circulating mature B (CD21+) cells is noted in 6-month old (PNM6) minipigs when compared with 2-month old (PNM2) but was no longer evident after a further four months (PNM6 minipigs).

- Treatment with CsA at 10 mg/kg/day over 4 weeks did not induce significant changes in lymphocyte and monocyte counts. These changes were no longer seen or were no longer statistically significant between groups.

- TCR cell counts were not affected by treatment with CsA.

- The proportion of circulating total, differential white blood cell counts, cytotoxic-T (CD8+) cells, double CD4+CD8+ positive T-lymphocytes, B cells and γδ-T-lymphocytes was generally comparable from PNW4 to PNM6 Göttingen minipigs. There were no gender differences at any age. The increase in mean γδ-T-lymphocyte cell counts noted in PN24 control animals was influenced by high interindividual variability.

- There was a significant reduction in proliferative response to ConA stimulation.

- The decrease in lymphocyte counts noted in the CsA-treated group correlated with lower mature B cells, helper-T and cytotoxic-T cells. The changes in immune cell subtypes were no longer seen or were no longer statistically significant between the previously treated water- and CsA-groups. 4 weeks or 5 months after the end of the treatment period.

- NK and γδ-TOR cell counts were not affected by treatment with CsA at the dose level of 10 mg/kg/day in juvenile Göttingen minipigs.

- Inter-individual variations were particularly noted for neutrophils and CD8+ and γδ-TOR lymphocytes.

3.2 Lymphoproliferation test – Figure 3

- A marked reduction (p < 0.05) in proliferative responses to Con A stimulation was observed in juvenile Göttingen minipigs treated orally with CsA 10 mg/kg/day over 4 weeks. There was no evidence of gender difference in lymphoproliferation parameter.

- The reduction in proliferative response was statistically significant (p < 0.01) for weeks after the end of the treatment period (PN24) but was no longer evident after a further four months (PNM28).

CONCLUSIONS

A trend towards higher peripheral mature B cell counts was observed in 6-month old minipigs when compared with younger animals whereas circulating helper-T cell proportion was lower in 2- and 6-month old minipigs than in 4-week old piglets. The proportion of other main peripheral immune cell populations and the lymphoproliferation response to ConA stimulation were comparable between age (2-week, 4-week, 2-month and 6-month old minipigs) and between males and females.

The immunosuppressive effect of cyclosporin A (CsA) was demonstrated in 2-week and 6-week old juvenile Göttingen minipigs, as shown by reduced white blood cell counts, associated mainly with lower mature B cells, helper-T and cytotoxic-T cells, as well as a significant reduction in proliferative response to ConA stimulation.

Cyclosporin changes in immune cell counts were no longer seen or were no longer statistically significant, 4 weeks or 5 months after the end of the treatment period (i.e. 2- and 6-month old minipigs). The proliferative response to ConA stimulation was still reduced in previously treated CsA-minipigs despite the 4-week treatment free period, although much less pronounced than during treatment.

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