IMMUNOGENICITY TESTING OF ADALIMUMAB AND INFLIXIMAB IN GÖTTINGEN MINIPIGS®

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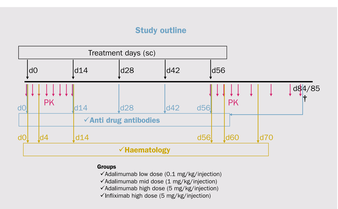
AS THE MINIPIG IS CURRENTLY CONSIDERED TO BE A USEFUL ALTERNATIVE SPECIES FOR SAFETY EVALUATION OF (BIO)PHARMACEUTICALS WE EXPLORED THEIR POSSIBILITIES FOR IMMUNOGENICITY TESTING. IN THIS STUDY THE POTENTIAL IMMUNOGENICITY OF TWO TNFα BLOCKERS, ADALIMUMAB AND INFLIXIMAB, BOTH CLINICALLY USED IN CHRONIC INFLAMMATION DISORDERS, IS EVALUATED.

Adalimumab is a recombinant human IgG1 monoclonal antibody, whereas Infliximab is a chimeric IgG1κ monoclonal antibody produced by a recombinant cell line from a genetically engineered form of the mouse monoclonal antibody prototype. These pharmaceuticals differ in biological activity in minipigs as Adalimumab is active and Infliximab is inactive.

STUDY OUTLINE

Minipigs (4 females/group) were treated subcutaneously every other week for 8 weeks with low dose (0.1 mg/kg), mid dose (1 mg/kg) or high dose (5 mg/kg) Adalumimab or high dose (5 mg/kg/day) Infliximab, followed by a 4 wk recovery period. Clinical signs, injection site reactions, body weight, haematology and the weight and pathology of several organs were used as criteria for disclosing possible adverse effects.



RESULTS

Clinical observations and body weights

No treatment related clinical signs were observed. Minipigs were not significantly affected in their growth (expressed as body weight) by Adalimumab or Infliximab treatments (data not shown).

Haematological analysis

On several occasions the % monocytes in the high dose Adalimumab group were higher compared to the low dose Adalimumab group, but in general no consistent and/or dose related changes in haematology parameters were observed between treatment groups.

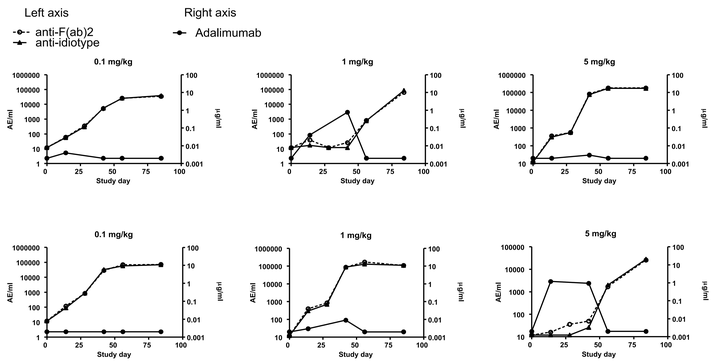


Figure 1: Individual levels of Adalimumab and anti-adalimumab antibodies in female minipigs after subcutaneous treatment on days 0, 14, 28, 42 and 56. Grossly all antibodies detected are directed towards the idiotype part of Adalimumab. Two representative animals from each group are depicted.

Table 1: Results of PK analysis of Adalimumab in minipig plasma after the first (day 0) and last (day 56) subcutaneous injection (n=4 unless otherwise depicted).

Adalimumab dose	0.1 MG/KG	1 MG/KG		5 MG/KG	
	Day 0	Day 0	Day 56	Day 0	Day 56
Cmax (µg/ml)	1.2 ± 0.1	7.2 ± 1.0 (n=3) 10 (n=1)	n.d 18.8 (n=1)	40.3 ± 4.7 (n=3) 49 (n=1)	n.d. 25.3 (n=1)
Tmax (days)	3 ± 1	7.3 ± 1.2 (n=3) 8.0 (n=1)	n.d 4 (n=1)	6.7 ± 1.2 (n=3) 6.0 (n=1)	n.d. 2 (n=1)
T1/2 (days)	0.70 ± 0.1	1.07 ± 0.6 (n=3) 8.24 (n=1)	n.d 6.22 (n=1)	0.73 ± 0.4 (n=3) 0.68 (n=1)	n.d. 1.56 (n=1)
AUC (0-∞) (day*µg/ml)	8.50 ± 1.3	53.3 ± 3.9 (n=3) 176 (n=1)	n.d. 295 (n=1)	347 ± 10 (n=3) 389 (n=1)	n.d. 120 (n=1)
Vz_F (L/kg)	0.012 ± 0.00	0.029 ± 0.01 (n=3) 0.067 (n=1)	n.d 0.030 (n=1)	0.015 ± 0.01 (n=3) 0.013 (n=1)	n.d. 0.094 (n=1)
Cl_F (L/kg*day)	0.012 ± 0.0	0.019 ± 0.0 (n=3) 0.006 (n=1)	n.d. 0.003 (n=1)	0.014 ± 0.0 (n=3) 0.013 (n=1)	n.d. 0.042 (n=1)

Adalimumab levels and anti-adalimumab antibodies (AAAs)

Eleven out of 12 animals treated with different levels of Adalimumab developed a clear AAA-response. . A clear correlation between the presence of high levels of AAA and low levels of Adalimumab in the serum of these animals can be observed (figure 1). By blocking the idiotype part of Adalumimab during the ELISA for AAA-detection, it was confirmed that the antibodies formed are directed towards the idiotype part of the Adalimumab (data not shown). In 6 out of 8 animals treated with mid- or high-dose Adalimumab, the Adalimumab is cleared from the blood so quickly that PK analysis is not applicable (table 1).

Infliximab levels and anti-infliximab antibodies

Infliximab levels are high during the whole study period from first injection onwards (figure 2). In the serum of these animals no anti-drug antibodies (ADA) can be found. PK analysis shows that Cmax, Tmax and T1/2 are comparable after the first and the last injection of Infliximab (table 2).

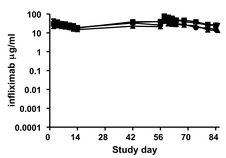


Figure 2: Levels of Infliximab in plasma of individual female minipigs after subcutaneous treatment on days 0, 14, 28, 42 and 56.

Table 2: Results of PK analysis of Infliximab in minipig plasma (n=4) after the first (day 0) and last (day 56) subcutaneous injection.

Infliximab dose	5 MG/KG	
	Day 0	Day 56
Cmax (µg/ml)	32.5 ± 7.9	57.4 ± 17.8
Tmax (days)	2.5 ± 1	2.5 ± 1
T1/2 (days)	12.8 ± 4.1	19.6 ± 2.0
AUC (0-∞) (day*µg/ml)	661 ± 129	1527 ± 472
Vz_F (L/kg)	0.139 ± 0.028	0.099 ± 0.031
Cl_F (L/kg*day)	0.008 ± 0.002	0.004 ± 0.001

DISCUSSION

As no clinical signs and (histo)pathological changes were observed and normal growth of the animals was observed, it can be concluded that the dosages of Adalimumab and Infliximab used were not in the toxic range for the minipig. Adalimumab elicited an anti Adalimumab antibody (AAA) response in 11/12 animals which was capable to drastically decrease the serum levels of Adalimumab. The majority of AAA was directed towards the idiotype part of the Adalimumab. This is comparable to the human situation in which AAAs, detected in 17% of patients, also are directed towards the idiotype part of the Adalimumab. Preclinical studies with Adalimumab in cynomolgus monkeys showed that at low dose levels (≤10 mg/kg) 100% of monkeys developed an AAA-response.

In minipigs treated with high dose Infliximab no evidence of immune-mediated clearance can be observed. In contrary, in humans Infliximab is highly immunogenic (10-61% of patients treated with Infliximab develop ADA to Infliximab after i.v. treatment). This difference might be related to the lack of TNFα-recognition in the minipig, as the same is observed in cynomolgus monkeys (in which Infliximab is not biologically active) versus chimpanzees (in which Infliximab is biologically active). It might be of interest to study whether or not ADA production is linked to (the lack of) biological activity of a compound in the species studied.

CONCLUSION

In conclusion, the immunogenicity results obtained in the minipig using the monoclonal antibodies Adalimumab and Infliximab indicate that the minipig has potential to serve as an alternative non-rodent model to study immunogenicity of biopharmaceutical products for human use. However, a prerequisite might be that the monoclonal antibody tested should be cross-reactive with the target antigen in pig which is also a regulatory requirement for safety testing of biopharmaceuticals.

FUTURE DEVELOPMENTS

For further validation and acceptance of the minipig as an alternative species for immunogenicity testing, more studies with minipigs should be performed.

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