IMMUNOGENICITY TESTING OF KINERET® IN GÖTTINGEN MINIPIGS®

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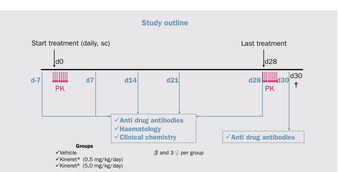
THE AIM OF THIS PROJECT IS TO INVESTIGATE THE FEASIBILITY OF GÖTTINGEN MINIPIGS® AS ALTERNATIVE SPECIES FOR IMMUNOGENICITY TESTING OF BIOPHARMACEUTICALS. IMMUNOGENICITY IS A PRIMARY ISSUE OF CONCERN FOR HUMAN SAFETY AND IN PARTICULAR EFFICACY OF BIOPHARMACEUTICALS.

Immunogenicity testing is focused on the detection and characterization of anti-drug antibodies (ADAs) and the determination whether these ADAs are neutralizing antibodies (NABs), as NABs may affect the pharmacokinetics (PK) of the drug. It is clear however that animal studies are not considered relevant to predict immunogenicity in humans. Still, the selection of the minipig to explore their possibilities for immunogenicity testing of biopharmaceuticals, especially to assist in the interpretation of safety studies, is based on the improved knowledge that various physiological aspects of Göttingen Minipigs® resemble more the human situation than other animal species[1]. It is expected therefore that in the near future the safety testing, including immunogenicity testing of biopharmaceuticals in minipigs will increase as an alternative for non human primate studies, which use will be limited or restricted.

STUDY OUTLINE

Minipigs (3 ♂ and 3 ♀ per group) were treated subcutaneously daily for 29 consecutive days with either vehicle, low dose Kineret® (0.5 mg/kg/day) or high dose Kineret® (5 mg/kg/day)[2]. This recombinant human IL-1R antagonist shows biological activity in the minipig. At several time points blood samples were collected for PK analysis and ADA determinations.



RESULT

Clinical observations

In some animals (♂ and ♀) treated with Kineret® redness and subcutaneous nodules were observed around the injection sites. These observations were temporary and lasted only a few days. No other treatment-related clinical signs were observed.

Body weight (Figure 1)

All animals showed growth during the study, which was not significantly affected by the treatment. Only in high-dose Kineret® treated males, body weight was significantly increased on day 28 only.

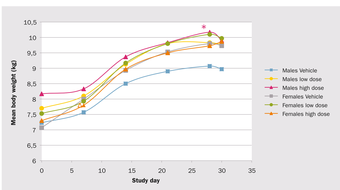


Figure 1

Haematology and clinical chemistry

No consistent changes were observed as a result of Kineret® -treatment in RBC and coagulation parameters, total and differential WBC counts and clinical chemistry parameters (not shown).

Anti-Kineret® antibodies (Figure 2)

All animals treated with Kineret® showed ADAs from day 14 onwards, whereas in the vehicle treated animals no ADAs to Kineret® were detected. The low dose treated males produced on the average less ADA than the high dose treated males. In females no dose-effect relation was observed in ADA production.

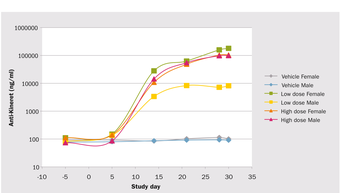


Figure 2: Group mean levels of ADA in male and female minipigs after daily subcutaneous Kineret®-treatment.

Pharmacokinetics of Kineret® (Figure 3, Table 1)

Plasma concentrations of Kineret® were determined prior to and several time points after the first (day 0) and last (day 28) dose.

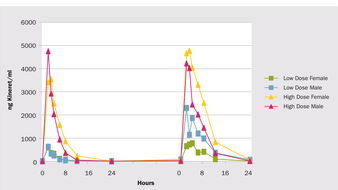


Figure 3: Group mean plasma Kineret® concentrations in male and female minipigs prior to and several time points after the first (day 0) and last (day 28) injection.

On day 28, both Cmax and AUC were higher for both males and females of the low dose group compared to day 0. At the high dose the AUC was also higher for both males and females compared to day 0. Given the large inter-individual differences in some calculated pharmacokinetic data no remarkable sex-differences were observed in the high dose Kineret® groups for most PK parameters. Both at the low and high Kineret® dosing no indications were found that anti-Kineret® antibodies neutralized Kineret® plasma concentrations. The increased internal dose at day 28 compared to day 0 was mainly due to increase of Cmax instead of a decrease in half-life (elimination)

DISCUSSION

Table 2: Comparison between minipig, non-human primate and human studies.

	ADA	ADA NEUTRALIZING	TMAX	T1/2
Minipigs	Yes	Probably not (work in progress)	± 2h	1.1-2.3h
Non human primates	Yes	No	± 2h	1.9-2.7h
Humans	3.8-57.2% of patients	In 2% of patients	3-7h	4-6h

Table 1: Results of PK analysis of Kineret® in minipig plasma

Low dose group (0.5 mg/kg/day)

		FEMALE DAY 0	FEMALE DAY 28	MALE DAY 0	MALE DAY 28
Cmax	(ng/ml)	594.6	770	623	2219
Tmax	(h)	2	4	2	2
T1/2	(h)	1.72	2.31	1.87	1.17
Lz	(h-1)	0.40	0.30	0.37	0.59
$AUC_{0-\infty}$	(ng h/ml)	2081	5044	1858	12618

High dose group (5 mg/kg/day)

		FEMALE DAY 0	FEMALE DAY 28	MALE DAY 0	MALE DAY 28
Cmax	(ng/ml)	3540	4698	4747	4200
Tmax	(h)	3	3	2	2
T1/2	(h)	1.19	1.05	1.35	1.13
Lz	(h-1)	0.58	0.66	0.51	0.62
$AUC_{0-\infty}$	(ng h/ml)	18489	33162	15689	22674

CONCLUSION

In summary it can be concluded that comparable results were obtained in respect to the immunogenicity and PK of Kineret® in minipigs and non human primates and that these observations are in agreement with those obtained in humans.

FUTURE DEVELOPMENTS

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

TNO innovation for life

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Ellegaard GÖTTINGEN MINIPIGS A/S

1 *Minipigs as model for the toxicity testing of new medicines and chemicals: an impact assessment, A special issue of the RETHINK project, Editor Roy Foster, J Pharmacol and Toxicol Methods, 62: 3; 157-242, 2010.*
2 *For comparison: human dose is 100 mg/day, corresponding to approximately 1.4 mg/kg/day*