Ustekinumab pharmacokinetics after subcutaneous administration in pig

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Abstract

Background. Due to multiple similarities in the structure and physiology of human and pig skin, the pig model is very useful for biological drugs testing after subcutaneous administration. The differences between subcutaneous injection sites could have significant impact on the absorption phase and pharmacokinetic profiles of biologic drugs. The aim of the current study was to analyse pharmacokinetics and selected biochemical and hematological parameters after a single subcutaneous administration of ustekinumab – human therapeutic monoclonal antibody, in regular pigs and compare parameters following administration into external or internal regions of the inguinal fold. The subjects were 12 healthy female large white piglets. Each group received a single dose of ustekinumab given in a 1 mg/kg subcutaneous injection into the internal inguinal fold or external part of the inguinal fold. **Results.** An analysis of hematological parameters showed that a single subcutaneous dose of ustekinumab at dose 1 mg/kg of body weight in pigs does not cause changes in the hematological parameters that go beyond the physiological limits. The differences in absorption rate between the internal inguinal fold or external part of the inguinal fold were not significant. However the time of maximal concentration, clearance, area under the curve calculated between zero and mean residence time and mean residence time between groups were substantially different ($p>0.05$). The relative bioavailability after external part of the inguinal fold administration was 40.36% lower than after internal inguinal fold ustekinumab administration. **Conclusions.** Breeding pig has been shown to be a relevant pharmacokinetics model to study subcutaneously administered ustekinumab.

Introduction

The pig model was investigated in pharmacometry and toxicology of small molecules and biologics [1–4]. The value of the pig model has been shown in many studies [5–9]. Due to multiple similarities in the structure and physiology of human and pig skin, this model is very useful for subcutaneous (SC) formulations testing [10]. Moreover, the Fc receptor of pigs, critical for the elimination half-life of therapeutic monoclonal antibodies (mAb), is similar to the Fc human receptor [11]. Therefore, in pig pharmacokinetic studies with SC administration, the absorption process and general pharmacokinetics profile could represent a relevant pharmacokinetic model for preclinical development [12, 13]. In pig model pharmacokinetics studies mostly inguinal and scapular regions are used for SC mAbs administration [9]. Different SC injection sites could have a significant impact on the shape of the absorption phase and pharmacokinetics of biologics [14–17]. The key limiting factor related to absorption is lymphatic drainage and presence of nearby lymph nodes [18]. The site of administration in the case of SC studies may affect not only pharmacokinetics of biological drugs but also their variability [19]. This explains why it is difficult to optimize the animal pharmacokinetics model of mAb's with SC administration.

Ustekinumab is a fully humanized IgG1 monoclonal antibody (mAb) with high affinity to human cytokines IL–12 and IL–23. Neutralization of biological activity of these cytokines is used for plaque psoriasis and psoriatic arthritis treatment in humans [20]. Intravenous and SC administration of

ustekinumab has an adequate pharmacokinetics profile, and effectiveness and safety of the administered drug is established [21]. In humans the recommended injection sites are the upper thigh or the area around the belly (abdomen) at least 5cm away from the navel (belly button). After abdominal SC injection in patients, biological drugs are absorbed into inguinal, iliac, and lumbar lymph nodes [22]. Inguinal fold of pigs is drained among others by afferent vessels of the inguinal lymph node [23].

The aim of the current study was a pilot analysis of PK and selected biochemical and hematological parameters after a single SC administration of ustekinumab in regular pigs and comparison between administration, into external or internal region of inguinal fold.

Materials And Methods

Animals

The subjects were 12 healthy female large white piglets weighing between 15.0 and 20.0 kg aged 45 ± 10 days. The study was approved by the ethics committee of the University of Life Sciences, Lublin (51/2017). Source of piglets was breeding farm of University of Life Sciences in Lublin. The water *ad libitum* and food were available throughout the study. The animals were divided into 2 groups ($n = 6$). Each group received a single dose of ustekinumab (Stelara[®], Janssen-Cilag International NV) given in a single 1 mg/kg SC injection into the internal inguinal fold in the first group (IF) or external part of the inguinal fold in the second group (EF). Before (at time 0) and after drug administration, blood was sampled from the jugular vein (2 mL) at intervals of 6, 12, 24, 36, 48, 60, 72, 84, 96, 120, 144, 168, 288, 384 and 432 h (18 days) after injection into heparinized tubes using a vacutainer (BD Vacutainer[®] Safety-Lok) for pharmacokinetics and anti-drug-antibodies (ADA) analysis. After the study animals were used for breeding only.

Hematological and biochemical parameters

The blood samples were analyzed for basic parameters in the same sampling points used for pharmacokinetics analysis. The analyzed parameters cover: cholesterol, bilirubin, urea, creatinine, phosphorus, calcium, WBC—number of white blood cells; LYM—number of lymphocytes; MID—number of other types of WBC not classified as lymphocytes or granulocytes; GRAN—number of granulocytes; RBC—number of red blood cells; HTC—hematocrit; HGB—hemoglobin; MCV—red blood cells corpuscular volume; MCH—red blood cells mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin concentration; RDW_a—absolute red blood cell distribution width; RDW—red cell distribution width; PLT—platelet count; MPV—mean platelet volume. All parameters were calculated using an automated hematology analyzer—Abacus Junior Vet (Diatron Group, Hungary).

Immunochemical analysis

The production of pig-anti-ustekinumab antibody was done with an additional two animals. The procedure was approved by the Local Ethical Committee for Animal Experiments in Wroclaw (resolution No. 07/18, 21.02.2018). Two pigs aged 2.5 months were immunized intramuscularly 4× every 2 weeks with 100 µg of ustekinumab in 0.5 mL of saline emulsified immediately before injection with 0.5 mL of Freund's Adjuvant, Incomplete (Sigma, F5506). Before each next injection the vein blood sample was taken from the *vena cava cranialis*. Two weeks after the last injection about 200 mL of the blood was taken using a 1.6 mm needle and 20 mL syringes. IgG from pig serum was purified by ion-exchange chromatography [24]. The IgG rich fraction of hyperimmune pig anti-ustekinumab serum was precipitated using ammonium sulphate. The precipitate was dialyzed against 0.05M phosphate buffer pH 7.0. The supernatant (about 180 mL ($A_{280} = 55$)) was loaded onto a DE Cellulose (DE52) column (120 mL) equilibrated with the same 0.05M phosphate buffer pH 7.0. The non-bound fraction (210 mL, $A_{280} = 28.5$) was recognized as porcine IgG. Production of affinity-purified IgG anti-ustekinumab was performed by subsequent steps. 0.4 mL of ustekinumab solution (36 mg) was dialyzed against 0.05M carbonate buffer containing 0.5 M NaCl to remove low-molecular substances possessing free amino-groups, prior to binding with activated Sepharose 4B (CNBr Activated Sepharose® 4B) according to manufacturer recommendations (Sigma-Aldrich). 50 mL of pig IgG containing ustekinumab-antibody formerly purified by ion exchange chromatography was bound to ustekinumab coupled to a Sepharose 4B column (3 mL). The column was washed with 0.05M phosphate buffer pH 7.0 containing 0.1 M NaCl, then with 0.05M phosphate buffer pH 7.0 containing 0.7M NaCl, and then with distilled water. The fraction containing affinity-purified porcine IgG anti-ustekinumab was eluted using 0.1M glycine-HCl buffer pH 2.2. Harvested fractions were neutralized using 1M NaOH and 1M Tris base. Approximately 5.5 mL of affinity-purified IgG was obtained ($A_{280} = 5.5$). The preparation was dialyzed against a solution containing 0.5M NaCl and 0.05M NaHCO_3 . Next, 10% (v/v) glycerol was added and the antibody was frozen until required. Conjugation of affinity purified porcine IgG anti-ustekinumab was performed with horseradish peroxidase [25]. 2 mL (10 mg) of affinity purified pig IgG anti-ustekinumab antibody solution was thoroughly dialyzed against 0.05M sodium carbonate buffer pH 9.5 containing 0.5M NaCl to remove Tris. Next, the preparation was dialyzed against 0.05M sodium carbonate buffer pH 8.0 containing 0.5M NaCl (+4°C). Then the contents of the dialysis bag were mixed with ammonium sulphate at (+4°C). Following centrifugation the supernatant containing porcine anti-human IgG1 HRPO conjugate was retained. Immune reactivity of the HRPO-conjugated porcine IgG antibody with ustekinumab was confirmed using direct ELISA. The wells of the polystyrene microplate (Nunc, Maxisorb, 96 wells) were coated with decreasing concentrations of a double dilutions series of ustekinumab (start well 4 mg/L final well 0.063 mg/L). The control wells were coated with bovine serum albumin (Sigma, A2153). The obtained HRPO-conjugated antibody was diluted 1:1000, 1:2000, 1:5000, 1:10000. Affinity purified porcine IgG anti-ustekinumab was coated on a microplate (100 µL, 5 mg/L per well); the microplates were then washed 3×5 minutes with PBS containing 0.05% Tween 20 (PBS-T), then the swine blood serum samples (diluted 1:100, 100 µL/well) were added in duplicate and incubated for 2h. After washing, HRPO-conjugated porcine IgG anti-human recombinant IgG1 was added (diluted 1:100000, 100 µL/well). After the next washing, substrate solution (TMB, Sigma) was added (100 mL/well) and microplates were incubated in

the dark at room temperature for 10 min. The reaction was stopped with 50 μ L of 1M H₂SO₄ per well and read at λ = 450nm. A standard curve was prepared in duplicates with the following human recombinant IgG1 (ustekinumab) concentrations: 500; 250; 125; 62.5; 31.25; 15.625; 7.8125; 0 ng/mL. The intra assay CV was 2.887 %, all samples were tested within one assay. A standard curve was made in duplicate on every plate. All measurements were made in duplicate in one series. The determination of ADA two antibody HRPO-conjugates against porcine IgM (rabbit Anti-Pig IgM Secondary Antibody (HRP) LS-C59959), (LifeSpan BioSciences Ltd.) and E100–117 Pig IgM ELISA Quantitation Set (Bethyl Laboratories Ltd.) were negatively absorbed using ustekinumab coupled to the Sepharose 4B column described above. The antibody passing this column was determined as anti-pig IgM free of cross reactivity with ustekinumab. The porcine IgM antibody was determined by ELISA. Periods of microplate coating and respective steps and washings were performed as described above. Nunc Maxisorp Microplate wells were coated with ustekinumab (50 μ L per well, 5 mg/L). After washing, swine blood serum samples (diluted 1:100, 50 μ L/well) were added. After washing, anti-pig IgM HRPO produced as described above (diluted 1:100, 50 μ L/well) was added. After another washing, substrate solution (TMB, Sigma) was added (50 mL/well) and microplates were incubated in the dark at room temperature for 20 min. The reaction was stopped with 100 μ L of 1M H₂SO₄ per well and read at λ = 450nm.

Pharmacokinetics and statistical analysis

The pharmacokinetic analyses were performed based on raw data using Phoenix[®] WinNonlin[®] 8.1 software (Certara L. P., US) and ThothPro v 4.1 (ThothPro LLC). The calculations were based on the slope, height, area, and moment analysis after SC administration for two different body regions. Key pharmacokinetic parameters were included in the comparative analysis, i.e. k_a —absorption rate constant, k_{el} —elimination rate constant, $t_{1/2kel}$ —elimination half-life, t_{max} —time to reach C_{max} , C_{max} —maximal concentration, $AUC_{(0-t)}$ —area under the curve calculated between zero and the last sampling point, $AUC_{(0-C_{max})}$ —area under the curve calculated between zero and t_{max} , $AUC_{(0-MRT)}$ —area under the curve calculated between zero and $MRT_{(0-t)}$, $AUMC_{(0-t)}$ —area under the first moment curve calculated between zero and the last sampling point, $MRT_{(0-t)}$ —mean residence time calculated for the last sampling point, CL/F —clearance, V_z/F —apparent volume of distribution. Statistical analyses were carried out using Microsoft Excel 365 and GraphPad Prism[®] 6.01 software (GraphPad Software Inc., US). Predicted seroconversion time was calculated based on linear fit analysis, $y = a \times x + b$, where y —ADA concentration, a —slope, x —abscissa of arithmetic means per time point and b —is the y-intercept. Student t-test was used for IF versus EF comparison and two tailed Mann-Whitney test for ADA values analysis. Differences IF versus EF with $p < 0.05$ were considered as statistically significant.

Results

The comparison of pharmacokinetic profiles in both groups is shown in Figure 1. Differences in variability for individual concentration points between EF:IF group are shown in Figure 2. Only 4 out of 14 sampling

points have lower concentrations variability in IF than EF group. The comparison of pharmacokinetic parameters in both groups was shown in Table 1. Pharmacokinetics profile of ustekinumab after single SC administration was characterized by simple and predictable decline. It was shown that absorption phase in both cases (EF and IF) lasted 2–3 days. After 18 days from single subcutaneous dose last observed concentrations were still high and equaled 1308.18 ± 201.20 and 1850.73 ± 621.07 ng/mL for EF and IF respectively ($p > 0.05$). Assuming a linear decrease in concentrations described by the slope of the elimination rate constant, 99% elimination of the drug from blood plasma should take 102.6 ± 84.8 and 121.4 ± 36.7 days for EF and IF group respectively ($p > 0.05$). The first ADA concentrations were observed 144 h (6 days) after drug administration in both groups. The concentration of ADA in 144 h time point was 8.51 ± 4.49 (2 from 6 animals) and 0.04 ng/mL (1 animal from 6). ADA concentration in 432 h after drug treatment (day 18) was 40.19 ± 25.04 (5 out of 6 animals) and 20.55 ± 17.6 ng/mL (6 animals) for EF and IF group respectively ($p > 0.05$). Predicted seroconversion time was 10.96 and 73.04 h for EF and IF group respectively. Only $t_{\max}$ has lower variability in EF than IF group but the difference of RSD% values between groups was $< 5\%$. Differences in variability $< 5\%$ were also found in k_{el} , $AUC_{(0-t)}$ and Cl/F .

The comparison of selected biochemical and hematological parameters in both groups is shown in Table 2. Only 7 parameters out of 20 differ between the EF and IF group ($p < 0.05$). The physiological range of analyzed parameters has been exceeded only in the case of urea (EF and IF) and calcium level (EF and IF) (Table 2). Differences in variability of hematological and biochemical parameters between groups were $> 5\%$ only in the case of MID (5.79%), LYM (6.09%) and cholesterol (6.20%). In these three parameters variability in EF was higher than in the IF group.

Discussion

A small number of studies describing pharmacokinetic profiles of human mAb's in pigs after SC administration are cited in literature [6–9]. Until now published works have described selected pharmacokinetics parameters of trastuzumab, anakinra, etanercept and adalimumab (minipigs). The current study showed the applicability of a breeding pig model in ustekinumab preclinical studies. In general, the shape of pharmacokinetics profile of ustekinumab in the current study does not deviate from the observations made regarding trastuzumab, anakinra, etanercept and adalimumab in minipigs. The pharmacokinetics profile of ustekinumab after a single SC dose shows predictable and slow disposition. ADA, like in the case of other animal models, are also observed in the current study. The seroconversion time should have a strong impact on the observed $C_{\max}$. However, in the current model there was no impact of differences in the predicted seroconversion time (10.96 versus 73.04 h) on the $C_{\max}$ value ($p < 0.05$). Here, it could be hypothesized that in the first two days after a single ustekinumab dose the immune system of a pig has no influence on drug pharmacokinetics. The differences in k_a between EF and IF were not significant but ultimately the $t_{\max}$ between groups was substantially different ($p > 0.05$). To conclude, the rate (k_a) and amount of absorption ($C_{\max}$) until $t_{\max}$ time were the same in both groups and had no impact on drug bioavailability until $t_{\max}$. The differences in $C_{\max}$ and $AUC_{(0-C_{\max})}$ between groups were not significant but large differences between EF and IF group were provoked by a significant

difference in CL/F. The relative bioavailability after EF administration was 40.36% lower than after IF ustekinumab administration. Only changes of CL/F led to differences between $AUC_{(0-t)}$ and $AUC_{(0-MRT)}$ ($p < 0.05$). In general, CL/F in pig model was ≈ 12 times faster than in humans [21]. $t_{1/2kel}$ in the current pig model was around 12 days, which is close to the lower limit in humans that ranges from 15 to 32 days across various populations of different patients [21]. However it is noteworthy that V_z/F in the case of the pig model was only ≈ 2 times higher than in subjects with psoriasis [26]. The absorption process in the current model takes ≈ 2 days which is an advantage of the model. Such a time range allows accurate tracking of the absorption phase by an appropriate number of sampling points. The key mechanisms influencing the disposition of human mAb's in animal models cover numerous interactions with the immune system [18]. In the current study it was shown that in the absorption phase ($0-t_{max}$) interaction between ustekinumab and the pig immune system has no impact on pharmacokinetics parameters and does not generate any significant response by ADA formation. There are no notable differences in PK of ustekinumab between EF and IF group in the absorption phase. This means that changing the administration site does not significantly affect the absorption of the drug. However, differences in the administration site in the current study show strong impact on the immune system response. This response is very often delayed in its nature [27]. This has been observed with regard to parameters extending beyond the absorption phase ($MRT_{(0-t)}$, $AUC_{(0-t)}$, $AUC_{(0-MRT)}$, $AUMC_{(0-t)}$, CL/F) and differences between groups related to selected hematological parameters. The xenogenic immunoglobulins induce the humoral immune response, and immune complexes are involved in their rapid removal from the blood of recipient. In a former self-study, yolk immunoglobulin (IgY) added to first colostrum or used as colostral IgG replacer in calves was absorbed to the blood, but disappeared within 2–3 weeks after feeding [28]. Anti-IgY antibodies were observed in the calves sera at 3rd week of life, remained detectable up to the end of the study (70 days of life) and their presence was associated with clearance of xenogenic proteins. The current study showed that the difference in administration site could have strong impact on ustekinumab concentrations variability (Fig. 2) and variability of calculated pharmacokinetics parameters. The observations made clearly indicate that variability of ustekinumab concentrations in EF group is lower than IF, especially in absorption and early distribution phase within the first 3 days after administration. However, in the whole group of PK parameters only t_{max} variability was lower in the EF group. An analysis of hematological parameters showed that a single SC dose of ustekinumab at dose 1 mg/kg BW in pigs does not cause changes in the hematological parameters that go beyond the physiological limits. At the same time, it should be noted that the proposed model is sensitive enough to show the impact of the administration site on selected hematological parameters while maintaining their physiological ranges. Ustekinumab had no negative effects on renal function [29]. This way it can be said that changes in urea do not reflect the impact of ustekinumab on kidney physiology. In the case of LYM, MID and GRAN the single dose of ustekinumab treated by EF and IF induces different effects with the IF group generating significantly higher values ($p < 0.05$). All three parameters still represent physiological values but prove that a different injection site could generate a different response related to immunocompetent cells. It should be emphasized that ustekinumab in immunotoxicity studies has no impact on circulating lymphocyte subpopulations, which is in line with the findings of the current study

[20]. The injection site has impact on RDWa values in both groups. That finding could be valuable in relation to the last results connecting cardiovascular disease risk in Crohn's with RDW and identification of RDW as a possible biomarker in psoriasis [30, 31]. In summary a breeding pig has been shown to be a relevant PK model to study SC administered ustekinumab.

Abbreviations

A_{280} —absorbance at 280 nm

ADA—anti drug antibodies

$AUC_{(0-C_{max})}$ —area under the curve calculated between zero and t_{max}

$AUC_{(0-MRT)}$ —area under the curve calculated between zero and $MRT_{(0-t)}$

$AUC_{(0-t)}$ —area under the curve calculated between zero and the last sampling point

$AUMC_{(0-t)}$ —area under the first moment curve calculated between zero and the last sampling point

BW—body weight

CL/F—clearance

C_{max} —maximal concentration

CV—coefficient of variation

EF—external inguinal fold

Fc—crystallizable region of mAb

GRAN—number of granulocytes

HGB—hemoglobin

HRPO—horseradish peroxidase

HTC—hematocrit

IF—internal inguinal fold

IgG—G class immunoglobulin

IL-12—interleukin 12

IL-23—interleukin 23

k_a —absorption rate constant

k_{el} —elimination rate constant

LYM—number of lymphocytes

mAb—monoclonal antibody

MCH—red blood cells mean corpuscular hemoglobin

MCHC—mean corpuscular hemoglobin concentration

MCV—red blood cells corpuscular volume

MID—number of other types of WBC not classified as lymphocytes or granulocytes

MPV—mean platelet volume

$MRT_{(0-t)}$ —mean residence time calculated for the last sampling point

PBS—Phosphate-buffered saline

PK—pharmacokinetics

PLT—platelet count

RBC—number of red blood cells

RDW—red cell distribution width

RDWa—absolute red blood cell distribution width

$RSD_{\%EF}$ —percent of relative standard deviation of concentrations after SC injection into external part of inguinal region

$RSD_{\%IF}$ —percent of relative standard deviation of concentrations after SC injection into internal part of inguinal region

SC—subcutaneous

$t_{1/2kel}$ —elimination half-life

t_{max} —time to reach C_{max}

V_z/F —apparent volume of distribution

WBC—number of white blood cells

Declarations

- Ethics approval and consent to participate

The study was approved by the ethics committee of the University of Life Sciences, Lublin (51/2017).

- Consent for publication Not applicable
- Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

- Competing interests

The authors declares that they have no competing interests.

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- Authors' contributions

All authors have read and approved the manuscript.

Tomasz Grabowski (1–5), Artur Burmańczuk (3,5), Rafał Derlacz (5), Tadeusz Stefaniak (3,5), Anna Rzęsa (3,5), Jacek Borkowski (3,5):

contributions to the conception (1) design of the work (2) the acquisition, analysis (3) interpretation of data (4) drafted the work or substantively revised it (5)

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Tables

Table 1 Pharmacokinetics parameters of ustekinumab (arithmetic mean; standard deviation) after single subcutaneous administration 1mg/kg BW into two different inguinal regions of a pig.

Parameter	External part of inguinal region	Internal part of inguinal region	<i>p</i> -value
k_a (h^{-1})	0.141; 0.147	0.102; 0.103	>0.05
k_{el} (h^{-1})	0.006; 0.004	0.003; 0.001	>0.05
$t_{1/2, kel}$ (h)	246.254; 203.61	291.355; 88.047	>0.05
t_{max} (h)	40; 11.314	62.4; 20.646	0.0347
C_{max} (ng/mL)	4302.855; 982.453	4329.768; 553.989	>0.05
$AUC_{(0-t)}$ ($h \times ng/mL$)	524279.414; 160129.037	879097.339; 235094.091	0.0096
$AUC_{(0-C_{max})}$ ($h \times ng/mL$)	962451.95; 463420.775	1728779.234; 697387.859	>0.05
$AUC_{(0-MRT)}$ ($h \times ng/mL$)	291264.047; 89329.841	433319.142; 85662.869	0.0193
$AUMC_{(0-t)}$ ($h^2 \times ng/mL$)	61272689; 44181081.751	122177749.4; 38626379.669	0.0286
$MRT_{(0-t)}$ (h)	103.99; 43.513	149.176; 19.283	0.0417
V_z/F (mL/kg)	316.202; 118.027	256.702; 63.403	>0.05
Cl/F (mL/h/kg)	1.267; 0.497	0.67; 0.233	0.0176

k_a – absorption rate constant; k_{el} – elimination rate constant; $t_{1/2kel}$ – elimination half-life; t_{max} – time to reach C_{max} ; C_{max} – maximal concentration; $AUC_{(0-t)}$ – area under the curve calculated between zero and the last sampling point; $AUC_{(0-C_{max})}$ – area under the curve calculated between zero and t_{max} ; $AUC_{(0-MRT)}$ – area under the curve calculated between zero and $MRT_{(0-t)}$; $AUMC_{(0-t)}$ – area under the first moment curve calculated between zero and the last sampling point; $MRT_{(0-t)}$ – mean residence time calculated for the last sampling point; Cl/F – clearance; V_z/F – apparent volume of distribution; *p*-value representing comparison of data between groups.

Table 2 Comparison of selected biochemical and hematological parameters (arithmetic mean; standard deviation) of pig blood after ustekinumab single subcutaneous administration 1mg/kg BW into two different inguinal regions.

Parameter	External part of inguinal region	Internal part of inguinal region	<i>p</i> -value
cholesterol (mg/dL)	102.296; 16.594	103.855; 10.411	>0.05
bilirubin (mg/dL)	0.017; 0.022	0.01; 0.012	>0.05
urea (mg/dL)	30.021; 6.216 [↑]	27.532; 5.452 [↑]	0.0054
creatinine (mg/dL)	1.025; 0.17	0.826; 0.14	<0.0001
calcium (mg/dL)	9.463; 1.423 [↓]	8.951; 1.269 [↓]	>0.05
phosphorus (mg/dL)	10.491; 1.184	11.255; 1.252	>0.05
WBC (×10 ⁹ /L)	22.836; 4.496	24.772; 4.097	0.0100
LYM (×10 ⁹ /L)	9.789; 1.819	10.09; 1.26	>0.05
MID (×10 ⁹ /L)	3.067; 0.775	3.343; 0.651	0.0279
GRAN (×10 ⁹ /L)	10.171; 2.922	11.419; 2.757	0.0134
RBC (×10 ¹² /L)	5.696; 0.489	5.599; 0.737	>0.05
HTC (%)	30.507; 2.871	29.284; 3.689	>0.05
HGB (g/dL)	10.098; 1.081	10.334; 1.453	>0.05
MCV (fL)	53.453; 1.941	53.266; 1.923	>0.05
MCH (pg)	17.759; 0.661	17.42; 0.832	>0.05
MCHC (g/dL)	33.236; 0.947	31.428; 1.125	0.0076
RDW (%)	20.441; 0.969	20.437; 1.404	>0.05
RDWa (fL)	36.892; 2.001	38.217; 2.684	0.0358
PLT (×10 ⁹ /L)	272.157; 93.548	274.355; 96.221	>0.05
MPV (fL)	7.372; 0.304	7.136; 0.241	>0.05

WBC – number of white blood cells; LYM – number of lymphocytes; MID – number of other types of WBC not classified as lymphocytes or granulocytes; GRAN – number of granulocytes; RBC – number of red blood cells; HTC – hematocrit; HGB – hemoglobin; MCV – red blood cells corpuscular volume; MCH – red blood cells mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; RDWa – absolute red blood cell distribution width; RDW – red cell distribution width; PLT – platelet count; MPV – mean platelet volume; [↑], [↓] – mean value higher or lower than physiological range ^{32, 33}; *p*-value representing comparison of data between groups.

Figures

Figure 1

Scatter plot representing two separate pharmacokinetics profiles of ustekinumab after subcutaneous injection into the external (rings) or internal (squares) part of inguinal region. Rings and squares represent observed concentrations, error bars represent means and standard errors of mean.

Figure 2

Difference between concentration variability for each time point of the concentration-time curve, external versus internal part of inguinal region. RSD%IF – percent of relative standard deviation of concentrations after SC injection into internal part of inguinal region, RSD%EF – percent of relative standard deviation of concentrations after SC injection into external part of inguinal region

Supplementary Files

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- [NC3RsARRIVEGuidelinesChecklist.pdf](#)