Original Research Article

Evaluation of pharmacokinetics and toxicology of biosimilar APZ001 antibody in *Macaca cynomolgus*

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Abstract

**Purpose:** To compare the pharmacokinetics of APZ001 antibody with those of cetuximab (Erbitux®) and to evaluate the toxicology of the former.

**Methods:** To evaluate cetuximab’s biosimilar APZ001, Crl:CD1(ICR) (CD-1) mice and Macaca fascicularis (cynomolgus monkey) were chosen for the studies on acute toxicity, chronic toxicity, pharmacokinetics in chronic toxicity and immunogenicity toxicity. The study also compared the pharmacokinetic parameters of APZ001 with those of cetuximab upon single and multiple drug administrations in cynomolgus monkeys.

**Results:** Pharmacokinetic parameters including maximum concentration (Cmax) and time to attain maximum drug concentration (Tmax), clearance rate and apparent volume of distribution of APZ001 were compared with those of cetuximab in both single and multiple administration studies. Difference of pharmacokinetics from weekly administration of APZ001 and cetuximab in cynomolgus monkeys was insignificant (p > 0.05), with relative bioavailability of 116.9%. Both APZ001-treated and cetuximab-treated CD-1 mice showed the same level of food intake and body weight. Hematological and serological data were similar from APZ001 antibody and cetuximab treatments, so were the acute and chronic toxicity. Weekly transfusion of APZ001 did not alter its pharmacokinetic parameters. The administered drug was hardly detected in the serum in the 31st and 37th week of recovery; no accumulation of drug was observed upon withdrawal.

**Conclusion:** APZ001 has extremely similar characteristics as cetuximab in terms of pharmacokinetics and toxicity.

**Keywords:** Cetuximab, Pharmacokinetics, Acute toxicity, Chronic toxicity, Immunogenicity, Biosimilar

INTRODUCTION

Aberrant overexpression and malfunction of epidermal growth factor receptor (EGFR) have been observed in many cancers, of which proliferation, apoptosis, angiogenesis and metastasis were mediated by it [1,2]. Upon EGF binding, EGFR forms hetero- or homo-dimers, leading to auto-phosphorylation and subsequently activating intracellular signaling...
transduction [3,4]. Suppressing cancer EGFR signaling via anti-EGFR monoclonal antibody (mAb) blocking is, therefore, an attractive therapeutic strategy [4].

Several mAbs-targeting cancer therapies have been approved by the Food and Drug Administration (FDA), including Erbitux (cetuximab) and Vectibix (panitumumab), which are used to treat colorectal cancer (CRC) and/or squamous cell carcinoma of the head and neck (SCCHN) by targeting EGFR [5,6]; Herceptin (trastuzumab) and pertuzumab, on the other hand, are used to treat gastric cancers by targeting EGFR2 [7,8]. It has been reported that a combined treatment of cetuximab and radiotherapy of the SCCHN increased the survival rate from 36 to 45 % during the phase III clinical trial (p = 0.018).

Due to the economic consideration, however, use of Erbitux and Herceptin are limited in the less developed countries. This work focused on developing a substitute of Erbitux and Herceptin for a more affordable therapy option. The cetuximab biosimilar antibody APZ001, of which the protein sequence and biological functions bear similarity to cetuximab, was investigated, with its preclinical pharmacokinetics and pharmacovigilance evaluated.

**EXPERIMENTAL**

**Reagents and drugs**

Cetuximab (used as the positive control) was purchased from Merck Serono (lot: 219265, imported drug license: S201300041). Saline buffer (0.9 %) was purchased from Kelun Pharmaceutical Co. Inc. (lot: B120518 F1); pentobarbital sodium from Sinopharma Chemical Pharmaceutical Co. Inc. (lot: B120518 F1); ketamine hydrochloride (0.1 mL/kg, 1.0 mL/kg, i.v.) was then injected to execute euthanasia. All animal-related experiments strictly followed the General principles for non-clinical safety technical reviews of therapeutic biologics and the Guide for the Care and Use of Laboratory Animals [9,10].

**Pharmacokinetic studies**

Thirty cynomolgus monkeys were randomly divided into five groups, with three males and three females in each group. Groups 1 to 3 received 7.5, 25 and 75 mg/mL of cetuximab biosimilar APZ001, respectively; group 4 received 75 mg/kg cetuximab for single pharmacokinetics analysis. APZ001 was administered for 4 weeks at a concentration of 25 mg/kg for multiple dose pharmacokinetics analysis. Blood samples were collected from groups 1 to 4 at 10 min, 20 min and 30 min (during administration); 1 h (administration endpoint); and at 4 h, 8 h, 24 h, 3 d, 5 d, 7 d, 9 d, 11 d, 14 d, 17 d, 21 d, 24 d and 28 d (after drug administration).

For group 5, cynomolgus monkeys received APZ001 weekly with their blood samples...
collected before the drug treatment (0 min), then at 10 min, 20 min and 30 min (during administration); 1 h (administration endpoint); and at 4 h, 8 h, 24 h, 3 d and 5 d (after drug administration). After the fourth weekly drug administration, blood samples were collected at 9 d, 11 d, 14 d, 17 d, 21 d, 24 d, 28 d, 30 d, 32 d, 35 d, 38 d, 42 d, 45 d and 49 d.

**Toxicity test**

To test the toxicity of biosimilar APZ001, 50 cynomolgus monkeys were randomly divided into 5 groups with sexual equality: group A, negative control; group B, cetuximab positive control; group C, low concentration of APZ001 administered; group D, medium concentration of APZ001 administered; group E, high concentration of APZ001 administered. Drugs were injected intravenously weekly after the initial administration, of which the concentration was at 12 mL/kg (week 1); the drugs were then delivered at 7.5 mL/kg in the following experiment weeks (weeks 2-26).

Leukocyte differential count, bone marrow white blood cell classification, and immunoglobin protein analysis were also performed. The clinical condition of the animal was also recorded daily, including the symptoms, start time, severity, duration and reversibility of toxicity. Weeks 5 and 11 were set as the recovery periods.

**Immunogenicity test**

Blood samples were collected for immunogenicity examination during the chronic toxicity test. IgA, IgE, IgG and IgM were measured by ELISA a week before the administration and then at week 4, 13, 26, 31 and 37. Anti-drug antibody was determined a week before the administration and then at week 1, 2, 4, 6, 8, 10, 12, 20, 26, 28, 31 and 37. Leukocyte differential count, bone marrow white blood cell classification and immunoglobin protein analysis were performed to monitor the change in the immunogenic indicators.

**Statistics analysis**

All statistics analysis was processed with Excel software. Pairs of samples were compared by F-test for equality of variances before using the t-test or U-test. Watson LIMS v.7.3.0.01 (Thermo Scientific Inc.) was used for serum drug concentration analysis. WinNonLin v 5.2.1 (Pharsight Inc.) software was used to calculate pharmacokinetic parameters.

### RESULTS

**Pharmacokinetics of APZ001 and cetuximab administered as a single dose**

$C_{\text{max}}$ and AUC values increased along with the administration concentration: the $C_{\text{max}}$ of 7.5, 25 and 75 mg/kg APZ001 were $183.08 \pm 10.89$, $642.98 \pm 49.97$ and $2132.31 \pm 229.53 \mu g/mL$, respectively, showing no statistically significant difference ($p > 0.05$); the AUC$_{\text{inf}}$ were $9661.09 \pm 1250.66$, $53608.67 \pm 7852.95$, and $183350.05 \pm 20360.84$ h$\cdot$μg/mL, respectively. Significant differences among three groups were noted ($p < 0.05$, Table 1) upon 7.5 and 25 mg/kg administration; the drug concentration showed a non-linear decrease, whereas the decrease was linear for the 75 mg/kg administration. The terminal elimination half-life of the low, medium, and high dosage groups were 6.29 ± 4.6, 69.11 ± 21.43, and 59.92 ± 46.69 h, respectively. The clearance rate (CL/F) was not significantly different between cetuximab and APZ001 at 25 mg/kg (Figure 1, $p > 0.05$) and was the same for the medium and high dose groups ($p > 0.05$, Table 1). The apparent volume of distribution (Vd) was also similar among the groups ($p > 0.05$, Table 1).

**Pharmacokinetics of APZ001 and cetuximab administered as multiple doses**

In group 5, administration of APZ001 at 25 mg/kg was repeated weekly. The $T_{\text{max}}$s of initial and terminal drug transfusion were $0.67 \pm 0.26$ and $0.92 \pm 0.20$, respectively. Initial and terminal $C_{\text{max}}$s were $668.61 \pm 56.05$ and $730.56 \pm 114.22 \mu g/mL$; AUC$_{(0-t)}$ were $37543.2 \pm 4484.23$ and $59742.48 \pm 33016.40$ h$\cdot$ng/mL; and AUC$_{\text{inf}}$s were
51676.29 ± 10262.62 and 59799.5 ± 33122.55 h•μg/mL, respectively. Initial and terminal elimination T1/2s were 84.81 ± 20.67 and 57.99 ± 11.65 h; clearance rates were 0.50 ± 0.10 and 0.60 ± 0.48 mL/h/kg; and Vd were 59.19 ± 8.84 and 47.01 ± 28.19 mL/kg, respectively.

Initial and terminal administrations were not significantly statistically different. The serum drug concentration quickly reached a stable level upon repeated administration with no drug accumulation detected. In conclusion, the multiple administrations resulted in similar outcomes for both APZ001 and cetuximab (p > 0.05); the pharmacokinetic parameters also showed little difference (p > 0.05) during the following treatment, with relative bioavailability 116.9%.

Toxicity
During the 26-week chronic drug administration, serum concentrations of APZ001 or cetuximab were tested (Figure 3) and biomarkers for liver function and kidney function were analyzed afterwards. Chronic treatment with 38/24 mg/kg cetuximab, 38/24 mg/kg APZ001 or 120/75 mg/kg APZ001 increased the levels of albumin (ALB) and globulin (GCB) but decreased the A/G ratio, which recovered in the first recovery period. Several doses increased the levels of alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), glutamic dehydrogenase (GLDH) and aspartate amino transferase (AST). GLDH level returned to the baseline after two recovery periods, while other biomarkers only after one. Although the levels of liver biomarkers were found to increase, no significant changes were observed in liver weight or liver pathological examination. The 38/24 mg/kg dose cetuximab.

Figure 2 shows the serum drug concentrations at each collection and the mean value of the group (n = 6). After four administrations, the drug accumulation ratio was 1.18 ± 0.07. Pharmacokinetic parameters of the initial and terminal administrations were not significantly different. The serum drug concentration quickly reached a stable level upon repeated administration with no drug accumulation detected. In conclusion, the multiple administrations resulted in similar outcomes for both APZ001 and cetuximab (p > 0.05); the pharmacokinetic parameters also showed little difference (p > 0.05) during the following treatment, with relative bioavailability 116.9%.

Table 1: Pharmacokinetic parameters of single dose injections

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>APZ001</th>
<th>Cetuximab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.5 mg/kg</td>
<td>25 mg/kg</td>
<td>75 mg/kg</td>
</tr>
<tr>
<td>T1/2a</td>
<td>h</td>
<td>26.29±4.6</td>
<td>69.11±21.43</td>
</tr>
<tr>
<td>T1/2b</td>
<td>h</td>
<td>54.02±12.15</td>
<td>95.85±17.3</td>
</tr>
<tr>
<td>Tmax</td>
<td>h</td>
<td>0.67±0.26</td>
<td>0.5±0.0</td>
</tr>
<tr>
<td>Cmax</td>
<td>mg/mL</td>
<td>183.08±10.89</td>
<td>642.98±49.97</td>
</tr>
<tr>
<td>AUC(0-t)</td>
<td>h•μg/mL</td>
<td>9658.61±1251.54</td>
<td>53582.58±7824.48</td>
</tr>
<tr>
<td>AUC(0-inf)</td>
<td>h•μg/mL</td>
<td>9661.09±1250.66</td>
<td>53608.67±7852.18</td>
</tr>
<tr>
<td>AUC(t-inf)</td>
<td>%</td>
<td>0.03±0.02</td>
<td>0.04±0.06</td>
</tr>
<tr>
<td>Vd</td>
<td>mL/kg</td>
<td>29.81±6.0</td>
<td>48.26±20.36</td>
</tr>
<tr>
<td>CLs</td>
<td>mL/h/kg</td>
<td>0.79±0.10</td>
<td>0.47±0.07</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>58.3±6.85</td>
<td>116.43±15.82</td>
</tr>
</tbody>
</table>

aRepresents efficacy half-life, b represents terminal phase half-life. a, p < 0.05; aa, p < 0.01; and aaa, p < 0.001 compared with Group 2. b, p < 0.05; bb, p < 0.01; and bbb, p < 0.001 compared with Group 3. c, p < 0.05; cc, p < 0.01; and ccc, p < 0.001 compared with Group 1. 

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Table 2: IgA level during 6-month chronic toxicity test

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (μg/mL)</th>
<th>D4, n=10</th>
<th>W4, n=10</th>
<th>W13, n=9</th>
<th>W26, n=7</th>
<th>W31, n=3</th>
<th>W37, n=2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>180.2±22.8</td>
<td>2.5±43.7</td>
<td>269.4±17.0</td>
<td>182.8±23.9</td>
<td>152.2±29.3</td>
<td>199.6±11.1</td>
<td></td>
</tr>
<tr>
<td>Cetuximab control</td>
<td>163.5±28.5</td>
<td>196.5±60.0</td>
<td>247.8±33.0</td>
<td>161.0±16.7</td>
<td>140.1±53.0</td>
<td>203.1±0.0</td>
<td></td>
</tr>
<tr>
<td>APZ001, low</td>
<td>162.3±17.8</td>
<td>194.8±30.1</td>
<td>252.5±20.7</td>
<td>172.4±27.9</td>
<td>210.8±21.5</td>
<td>193.1±58.6</td>
<td></td>
</tr>
<tr>
<td>APZ001, medium</td>
<td>187.9±50.1</td>
<td>204.1±24.7</td>
<td>256.9±29.6</td>
<td>168.8±20.6</td>
<td>191.1±16.4</td>
<td>223.1±26.3</td>
<td></td>
</tr>
<tr>
<td>APZ001, high</td>
<td>195.7±28.5</td>
<td>227.9±31.7</td>
<td>257.1±11.2</td>
<td>160.1±33.4</td>
<td>178.4±15.3</td>
<td>245.3±15.2</td>
<td></td>
</tr>
</tbody>
</table>

# Represents p < 0.05 compared with blank serum before drug treatment. D, days; W, weeks.

Table 3: IgG level during 6-month chronic toxicity test

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration (μg/mL)</th>
<th>D-4, n=10</th>
<th>W4, n=10</th>
<th>W13, n=9</th>
<th>W26, n=7</th>
<th>W31, n=3</th>
<th>W37, n=2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>12.6±2.0</td>
<td>14.0±3.7</td>
<td>17.0±1.2 #</td>
<td>12.6±1.2</td>
<td>10.4±1.6 #</td>
<td>13.7±0.3</td>
<td></td>
</tr>
<tr>
<td>Cetuximab</td>
<td>11.9±2.2</td>
<td>13.5±5.1 #</td>
<td>16.1±2.0 #</td>
<td>11.5±0.7</td>
<td>12.1±1.6</td>
<td>13.2±0.6</td>
<td></td>
</tr>
<tr>
<td>APZ001, low</td>
<td>11.3±0.8</td>
<td>14.2±2.6 #</td>
<td>17.3±1.3 #</td>
<td>11.7±1.2</td>
<td>12.8±0.3 #</td>
<td>14.1±0.1 #</td>
<td></td>
</tr>
<tr>
<td>APZ001, medium</td>
<td>11.6±1.4</td>
<td>14.3±1.6 #</td>
<td>18.3±1.9 #</td>
<td>11.6±0.8</td>
<td>11.7±0.4</td>
<td>11.2±0.3 *</td>
<td></td>
</tr>
<tr>
<td>APZ001, high</td>
<td>12.8±2.0</td>
<td>15.5±3.5</td>
<td>16.9±0.8 #</td>
<td>12.2±1.1</td>
<td>12.3±0.1</td>
<td>12.3±0.8</td>
<td></td>
</tr>
</tbody>
</table>

# represents p < 0.05 compared with blank serum; * represents p < 0.05 compared with negative control. D, days; W, weeks.

and APZ001 groups exhibited similar changes in levels of GCB, ALB, A/G, ALT, GGT, GLDH and AST, indicating the similar liver toxicity effects.

The levels of other kidney function markers, including urine nitrite, glucose, protein, bilirubin, urobilinogen, acetone bodies and white cell count, did not exhibit abnormality in all tested groups. The medium-dose APZ001 and cetuximab groups showed similar side effects (e.g. skin toxicities), which did not fully recover. Changes in pathology and lesions to other organs were not observed, nor were abnormalities in the cynomolgus monkeys’ body weight, rectal temperature, blood pressure, hematological coefficients and coagulation function. In conclusion, after the 6-month chronic toxicity test, various drug administrations did not cause significant changes, which included the rectal temperature, hematological and coagulation function, electrocardiogram, blood pressure, urea biomarkers, pathological examination, bone marrow and optical examination.

Immunogenicity

Serum immunoglobins IgA, IgE, IgG, and IgM were semi-quantified by ELISA according to the manufacturer’s instructions with slight modification. Quantification of serum IgA showed the results at eight time points were significantly different from the blank serum control (Weeks 4, 13, 31, and 37; p < 0.05; Table 2), but were the same as the positive control cetuximab group. During the week 13, a potential systematic error caused the results acquired from the blank group different from the blank serum. Quantification of serum IgG showed the results collected at eleven time points were different from the corresponding blank serum controls (both positive and negative) (Table 3, p < 0.05), indicating a systematic error. Several results obtained from the medium-dose cetuximab group showed differences from the negative control group (Table 2, p < 0.05). Quantification of IgE and IgM showed that the results were not significantly different from the negative groups.

DISCUSSION

In addition to internal organ epithelium cells, EGFR was also reported to be expressed in the human skin within keratinocytes, the follicular epithelium, sweat and sebaceous glands and...
capillaries of the dermis, [11,12]. Disturbance of EGFR signaling might result in hair follicle necrosis and alopecia [13,14]. Minor side effects were reported in an earlier acute toxicity study, such as hair disorganization and partly alopecia in the cynomolagus monkeys [15]. Additionally, blocking EGFR function caused pathological reactions, such as skin inflammation, folliculitis and rash, due to the presence of EGFR in sebaceous glands [16].

In this work, APZ001 was well metabolized and did not accumulate upon weekly i.v. administration for 6 months. In direct comparison with cetuximab, weekly administration of APZ001 did not induce any unprecedented adverse effects, while the predictable anti-EGFR–related side effects, such as skin rash, dehydration and liquid feces, occurred at acceptable levels and rates.

Administration of cetuximab and APZ001 increased the kidney weight and organ coefficient, but no pathological changes or lesions were observed. No apparent damage was observed in the kidneys upon pathological examination, nor were any hematological markers found. Previous study using fluorescent dye-labelled cetuximab also indicated moderate and acceptable toxicity to organs [17]. In addition, similar results were also reported by using a mixture of two biosimilars of cetuximab in the cynomolagus monkeys [18].

Long-term drug administration led to the accumulation of drug in the circulation system, thus enhancing the kidney excretion activity to metabolize the excessive drug; the enhanced kidney function was maintained over a long course of time, leaving it impossible to recover in two recovery periods.

Slight to moderate skin toxicity side effects occurred to some cynomolagus monkeys. It was speculated that complete blocking of EGF-EGFR function in skin tissue might cause epidermal cell death occurring at a faster rate than cell growth, thus inducing skin toxicity. Many clinical studies of cetuximab have shown a direct correlation between the severity of rash and efficacy of treatment [19,20]. Harandi and colleagues reported that patients with grade 3 rash had the highest survival rate during the treatment of cetuximab [21]. Administrations of APZ001 and cetuximab (38 and 24 mg/kg doses, respectively) induced similar toxicity reactions in skin and eyes, indicating that the chronic toxicity of APZ001 was within the safe range for human use.

**CONCLUSION**

The biosimilar, APZ001, showed similar properties to the positive control, cetuximab, including its pharmacokinetics, toxicokinetics, acute toxicity, chronic toxicity and immunogenicity toxicity. Thus, APZ001 antibody may show similar therapeutic effect to cetuximab.

**DECLARATIONS**

**Acknowledgement**

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**Conflict of Interest**

No conflict of interest associated with this work.

**Contribution of Authors**

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xiaofei Wang wrote the manuscript. Wei Yang, Jianmin Guo and Huqing Liang designed all the experiments and revised the manuscript. Xinyu Deng, Caiguo Ye, Yuankeng Huang and Xialing Lei performed the experiments.

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