Validation of Tumor bearing Nude Rat as a Model to study Anticancer Drug Pharmacokinetic/Pharmacodynamic (PK/PD) Relationships


Abstract#5411

INTRODUCTION

- A key issue in the clinical development of anticancer drugs is to have as much reliable information from relevant preclinical in vivo models.
- The Nude rat was selected as an appropriate model for human tumor profiling, assessment of delicate surgery, various administration routes of compounds, and repeated sampling.
- Paclitaxel (PXL) was chosen as a clinically active anticancer agent for which metabolism has been described in rodents and human (modified by cytochromes P450/CYP; 2C8 and 3A4).
- The objective of this study was to explore the PK/PD of PXL in a model of tumor bearing Nude rat treated by either intravenous (IV) bolus injection or continuous IV infusion.

EXPERIMENTAL METHODOLOGY

ACTIVITY OF CYP1A, 2B, 2E AND 3A IN MICE AND RATS

- Collection of liver, kidneys and intestines from healthy athymic Nude mice and Nude rats (Harlan, France).
- Preparation of microsomes following the method described by Auvray P. et al., (1).
- Measurement of protein concentration in microsomes with BioRad Protein Assay Kit II.
- Measurement of CYP activities as detailed in the table below:

<table>
<thead>
<tr>
<th>CYP isoform</th>
<th>Activity (pmole/min/mg)</th>
<th>Activity (pmole/m in/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A</td>
<td>Mouse Liver</td>
<td>Human Liver (S9)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>1000</td>
</tr>
<tr>
<td>CYP2B</td>
<td>Mouse Liver</td>
<td>Human Liver (S9)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>CYP2E</td>
<td>Mouse Liver</td>
<td>Human Liver (S9)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>CYP3A</td>
<td>Mouse Liver</td>
<td>Human Liver (S9)</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>2000</td>
</tr>
</tbody>
</table>

- Results are expressed in profile of production of protein.

PHARMACOKINETIC, METABOLIC, TOXICOLOGICAL, AND ANTITUMOR PROFILES OF PXL AFTER IV BOLUS INJECTIONS OR CONTINUOUS IV INFUSION IN TUMOUR BEARING NUDE RATS

- As in vivo experiment in Nude rats:
  - Organized intraperitoneal implantation in the femoral vein of Nude rats at D=26.
  - Whole body irradiation of cauterized Nude rats at D=19.
  - Subcutaneous inoculation of 10^5 tumor cells into nude mice at D=46.
  - Randomization (DR) of dosage when the mean (3SD) tumor volume reached 204 ± 20 mm^3.
- Vehicles of PXL composed of 2.5% Cremophor and 2.5% ethanol in saline for the same dose (20 mg/kg/day).
- Daily IV bolus injections of PXL at 5 mg/kg/day, from D0 to D3 (20 mg/kg/day) in the tail vein of rats anaesthetised with isoflurane.
- Continuous IV infusion of PXL at 20, 80 and 200 mg/kg/day between D0 – D4 via a catheter implanted in the femoral vein of rats coupled to a syringe-pump.

<table>
<thead>
<tr>
<th>Adm routes</th>
<th>Adm routes</th>
<th>Cumulative dose of PXL (mg/ml)</th>
<th>AUC (µg/ml h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D0 - D4</td>
<td>D0 - D4</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>D0 - D2</td>
<td>D0 - D2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>D0 - D1</td>
<td>D0 - D1</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

- Blood sampling collection via the tail vein after the first and third IV bolus injection and during the continuous IV infusion process of PXL (5 rats/sampling time).
- Collection of bile and urine samples of the Nude rats every 10 minutes via a drain at the end of treatments.
- Sacrifice and collection of SC tumors 6 hours after the end of treatments.

- Toxicological and antitumor activity profiles:
  - Monitoring of the rats body weight and survival between D0 and D30 (a).
  - Monitoring of tumor volumes between D0 and D30.
  - Determination of apoptosis in SC tumors with TUNEL assay.

- Pharmacokinetics analysis:
  - PXL pharmacokinetics in plasma at D0 and D3 for rats injected by IV bolus; rats
  - PXL pharmacokinetics in plasma between D0 to D5 for rats continuously infused by IV route.
  - Determination of PXL concentration in plasma by HPLC/MS/MS method using desoxim as internal standard.
  - Determination of PK parameter such as AUC, irreversible, F, equipotent analysis and C.
  - Identification of PXL metabolites in the bile and urine of rats using the Q3 Scan mode analysis by MS/MS.

- Confirmation of the structure of metabolites by simultaneous experiments using the Product Ion Scan and Precursor scans.

RESULTS

ACTIVITY OF CFPA, CFPE AND CFPA in Nude MICE AND RATS

- Activity of CFPA, CFPE and CFPA was significantly higher in liver of Nude mice than in Nude rats and human.
- Activity of CFPA, CFPE and CFPA was only detected in intestine of Nude rats.

PXL ANTITUMOR ACTIVITY AFTER REPEATED IV BOLUS INJECTION AND CONTINUOUS IV INFUSION IN CALU-6 TUMOR BEARING NUDE RATS

- The antitumor activity of PXL was associated with the maximal peak concentration and not with PXL exposure.
- PXL and four major metabolites of PXL were observed in the bile of tumor bearing Nude rats 20 minutes after IV bolus injection and at the end of IV infusion of PXL (we also suspected the presence of Baxtazol III as a fifth metabolite in the bile).
- PXL metabolites were only detected in bile of rats but not in urine (whatever the administration route).
- The results observed in tumor bearing Nude rats confirmed those previously observed in Sprague-Dawley rats.

CONCLUSIONS

- The Nude tumor bearing nude rat model was used to determine quantitatively the antitumor activity, toxicity, PK parameters and metabolism of PXL after IV bolus injections and continuous IV infusion.
- The Nude rat model appears to be appropriate for the clinical development of antitumor agents providing useful data for the determination of the optimal schedule of administration, both therapeutic and toxic effects, pharmacodynamics and metabolism.


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