The disposition, metabolism, and pharmacokinetics of a novel anti-tumor Agent, β-elemene, in Sprague-Dawley rats

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Abstract

Aim β-elemene, a natural sesquiterpene, is a cytotoxic agent of broad-spectrum anti-tumor activity. The metabolism, tissue disposition, excretion routes, and plasma pharmacokinetics of β-elemene after single i.v. administration have been investigated in male SD rats. Methods The metabolism of β-elemene was extensive and the structure of the metabolite in rat bile was determined by gas chromatography-mass spectrometry and NMR analysis. Results β-elemene was administered at three doses (50, 75, and 100 mg/kg) and a full pharmacokinetic profile was obtained. β-elemene was widely distributed in tissues and was rapidly eliminated. High permeability of β-elemene through the blood-brain barrier was found following i.v. administration based on the brain / plasma ratio. Following i.v. administration, the drug was primarily eliminated as metabolite and minimal unchanged drug. Cumulative fecal, biliary, and urinary excretion of β-elemene in rats was 0.61, 0.06, and 0.003%, respectively, of the administered dose (75 mg/kg) at 32 h after administration. Conclusions These results indicate that biotransformation may be the main elimination pathway of β-elemene.

Key words gas chromatography/mass spectrometry; β-elemene, pharmacokinetics; disposition; metabolism

Introduction

β-elemene, 1-methyl-1-ethenyl-2, 4-isopropenyl-cyclohexane, a natural sesquiterpene extracted from the roots and stems of Curcuma wenyujin Y.H et al C. Ling [1], is the main constituent of Elemene Emulsion, which is a broad spectrum anti-tumor agent[2,3] and is commercially available in the Chinese market. It prolonged the life time of tumor mice due to its cytotoxic effect, induction of apoptosis and regulating the expression of bcl-2 protein[4-6].

In the past, β-elemene has been a focus of attention in cancer research [3,7,8]. However literature data about the disposition and pharmacokinetics of β-elemene in animals is rare. Here, we conducted studies to determine the disposition and pharmacokinetics of the compound in male SD rat. This study represents the first detailed pharmacokinetic characterization of β-elemene.

Materials and Methods

Chemicals and drugs

β-elemene (purity: 98.8%) was provided by Yuan Da Pharmaceutical Company (Dalian, China). All other chemicals and solvents were purchased from commercial sources and used as received.
Animal experiments
The β-elemene emulsions used for all animal studies were prepared by Yuan Da Pharmaceutical Company. Male SD rats, purchased from the Experimental Animal Center of Dalian Medical University, ranging in age from 8 to 9 weeks and weighting approximately 200 g, were used for these studies. Rats were given free access to food and water.

Pharmacokinetic studies in rats
The single dose (50, 75 and 100 mg/kg) plasma pharmacokinetics of β-elemene was studied in rats after injection through the right femoral vein. Under light ether anesthesia rats were implanted with a PE-10 cannula into the left femoral vein and then allowed to recover for 1 h before dosing. Blood samples (five to six samples / time point) were collected via the catheter into heparinized glass tubes at 5, 15, 30, 45, 60, 90, 120, 180, 240, 300 to 360 min postdose after iv administration. Plasma was separated immediately by centrifugation (10 min, 4 °C, 3000 rpm) and were then processed immediately using the extraction procedure described below.

Tissue Distribution
For the tissue distribution studies, rats were decapitated at 15, 30, and 180 min after iv dosing of 75 mg/kg β-elemene and the trunk blood was collected into a heparinized glass tube. The whole blood was centrifuged at 3000 rpm for 10 min and the plasma was transferred for extraction. Immediately after blood collection, tissue samples were weighed and homogenized on ice with 3 or 10 volumes of ice-cold 20 mM phosphate buffer (pH 7.0) using a Polytron RDP homogenizer.

Plasma Protein Binding Determinations
Samples of rat plasma (0.5 ml) containing β-elemene at the concentration of 0.006% and 0.01% were dialyzed against phosphates buffer (0.5 ml), pH 7.4, for 24 h at 37 °C in a rotating dialyzer. Triplicate determinations were performed on pooled samples of male rat plasma for each concentration. After dialysis, the concentrations of drug in plasma and buffer were determined.

Excretion Studies
Four male Sprague-Dawley rats were administered i.v. a single dose of 75 mg/kg. After dosing, the animals were housed in individual metabolism cages designed for the separation and collection of urine and feces. Urine and feces were collected at 0-2 h, 2-4 h, 4-6 h, 6-8 h, 8-24 h, and 24-32 h, or 0-6 h, 6-8 h, 8-24 h, and 24-32 h, respectively.

Metabolism studies
A cannula was implanted surgically into the bile duct while the rats were under ether anesthesia. The biliary output was collected at 2 h intervals for the first 8 h, and then 8 h intervals after iv administration of β-elemene (75 mg/kg).

After collection, all the samples (plasma, tissue homogenates, bile, urine and feces) were processed immediately using the extraction procedure described below and analyzed.

Drug extraction: One milliliter of sample (plasma, tissue homogenates, bile, urine and feces) from individual rats was extracted with 2 ml of ether. The extract was evaporated to dryness in a 35°C water bath under nitrogen. The residue was reconstituted in 100 µl of CS₂ for GC analysis.

Analytical Techniques The gas chromatograph was a Shimadzu GC-7AG equipped with a fire ion detector. The stationary phase was 10% SE 30 on 80/100 Chromosorb WAW (Supelco, Bellefonte, CA), packed in a glass column (o.d. = 3.0 mm, length = 2.1 m). Nitrogen, at a flow of 40 ml/min, served as the carrier gas. The detector gases were hydrogen and air, at a flow rate of 40 and 400 ml/min, respectively. The analysis was performed under isothermal conditions at an oven temperature of 115 °C. Injector and detector temperatures were set at 210°C. An integrator model Chromatopac C-R2A Shimadzu was used. All sample extracts (plasma, tissue homogenates, bile, urine and feces) were analyzed by GC.

For metabolism studies, bile extracts were analyzed using a Hewlett Packard (HP; Waldbronn, Germany) 5890 Series II gas chromatograph.
combined with an HP 5972 A MS engine mass spectrometer. The GC conditions were as follows: splitless injection mode; column, SE 30 capillary (30 m × 0.2 mm i.d.), column temperature, 175 °C, carrier gas, helium; flow rate 1 ml/min; the MS conditions were as follows: full scan mode; EI ionization mode: ionization energy, 70 eV; ion source temperature, 170 °C.

1H NMR and 13C NMR spectra of the rat bile extracts were obtained using a Bruker DRX-400 NMR spectrometer. Samples were analyzed in CDCl₃, and chemical shifts are expressed relative to tetramethylsilane, which was added as the internal standard.

Standard calibration curves were constructed by analyzing a series of 1 ml aliquots of drug-free rat plasma containing 7 µg / ml of n-tridecane as internal standard and varying concentrations between 0.1 and 40 µg / ml of β-elemene. The concentration of drug in each unknown sample was determined by solving the linear calibration curve equation for each corresponding drug / internal standard ratio.

**Pharmacokinetics calculations**

Data manipulations and statistical calculations (mean ± S.D.) were performed in Excel software (Microsoft, Redmond, WA). For pharmacokinetics calculations, 3p87 (Section of Mathematics, Chinese Pharmacological Society) software was used. An appropriate model was chosen on the basis of the lowest sum of weighted squared residuals, the lowest Schwartz Criterion, the lowest Akaike's Information Criterion value, the lowest standard errors of the fitted parameters, and the dispersion of the residuals. F test was also used to discriminate between these hierarchical models (Gabrielsson and Weiner, 1997). The plasma concentration-time profiles of i.v. dosing β-elemene were fitted well to the follows two-exponential equation:

\[ C = Ae^{-\alpha t} + Be^{-\beta t} \]  

Where \( \alpha \) and \( \beta \) are the apparent rate constants, \( A \) and \( B \) are the corresponding time 0 intercepts, and \( t \) is time. The input data were weighted as the reciprocal of the square of the observed values, and the algorithm used for the fitting was the damping Gauss-Newton method.

The area under the plasma concentration-time curve (AUC) and area under the moment curve (AUMC) were calculated as

\[ AUC = \frac{A}{\alpha} + \frac{B}{\beta} \]  
\[ AUMC = \frac{A}{\alpha^2} + \frac{B}{\beta^2} \]

The plasma clearance (CL), distribution volume of the central compartment (V) and steady-state distribution volume (V₅₀) were calculated from Eqs. 4, and 5, respectively

\[ CL = \frac{\text{dose}}{AUC} \]  
\[ V_{50} = \frac{\text{dose} \cdot AUMC}{(AUC)^2} \]

**Statistical Analysis**

Statistical analyses of the experimental data were performed using Student's t test and one-way ANOVA.

**Results**

**Pharmacokinetics of β-elemene in Rats**

The mean plasma concentration versus time profiles for β-elemene after single intravenous injection to rats are presented in Fig. 2, and the summary of estimated pharmacokinetic parameters are listed in Table 1. After an i.v. dose the plasma concentration of intact β-elemene decreased biexponentially, and was eliminated from plasma with a terminal half-life of approximately 1 h. The mean plasma clearance of β-elemene was 0.046-0.058 liters/min/kg, with dose increasing from 50 to 100 mg/kg. The AUC₅₀ accounted for 85%, 90%, and 92% of AUCₙ at the dosage of 50, 75, 100 mg / kg respectively. The relationship between dose and AUC was evaluated from the value of AUCₙ. The AUC of β-elemene increased with increasing doses (AUCₙ versus dose; \( r^2 = 0.98 \)), indicating the linear pharmacokinetics from 50 to 100 mg/kg. The V₅₀ value was 1.9-2.6 liters / kg, which was much greater than the plasma volume, indicating that β-elemene highly distributed to tissues from plasma.

**Tissue distribution**

The mean tissue concentrations in rats after a single i.v. administration of 100 mg / kg β-elemene are shown in Fig. 3, and the tissue / plasma concentration ratio are summarized in Table 2. After administration to rats, the drug was observed to be distributed to all 10 tissues and plasma examined. Maximum levels of β-elemene were seen in all of the tissues by 15 min. The heart tissue exhibited the

The highest concentration. Other tissues with high concentration were kidney, spleen, fat, liver, brain, and lung. The concentrations of β-elemene in gastrointestinal and muscle were moderate. The tissues with the lowest exposure were plasma and testis. At 180 min postdose, liver tissue concentration of β-elemene lowered dramatically, while brain and fat displayed significant tissue accumulation of β-elemene in comparison of other tissues (see Table 2).

Fig.2. Plasma concentration-time curve of β-elemene after a single iv administration to rats. Each point represents the mean data from 5-6 rats. The vertical bars indicate the SD point. If not shown, the vertical bars are within the symbol.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>100 mg/kg</th>
<th>75 mg/kg</th>
<th>50 mg/kg</th>
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</thead>
<tbody>
<tr>
<td>A (µg/ml)</td>
<td>65.6±5.2</td>
<td>46.5±8.2</td>
<td>35.9±2.1</td>
</tr>
<tr>
<td>B (µg/ml)</td>
<td>6.2±2.0</td>
<td>8.6±4.2</td>
<td>2.4±1.2</td>
</tr>
<tr>
<td>α (/min)</td>
<td>0.048±0.008</td>
<td>0.067±0.016</td>
<td>0.055±0.009</td>
</tr>
<tr>
<td>β (/min)</td>
<td>0.0109±0.0009</td>
<td>0.013±0.004</td>
<td>0.0120±0.0018</td>
</tr>
<tr>
<td>t1/2 (min)</td>
<td>65.1±5.9</td>
<td>58.4±17.9</td>
<td>58.6±8.3</td>
</tr>
<tr>
<td>Vdss (liters/kg)</td>
<td>2.24±0.22</td>
<td>2.58±0.52</td>
<td>1.90±0.19</td>
</tr>
<tr>
<td>Cl (liters/min/kg)</td>
<td>0.046±0.004</td>
<td>0.056±0.004</td>
<td>0.058±0.005</td>
</tr>
<tr>
<td>AUC (µg·min/ml)</td>
<td>2200.6±186.2</td>
<td>1355.2±103.4</td>
<td>861.1±64.6</td>
</tr>
<tr>
<td>AUMC(µg·min²/ml)</td>
<td>107635.9±7866.4</td>
<td>62307.0±8329.6</td>
<td>28499.6±3029.7</td>
</tr>
</tbody>
</table>

### Plasma Protein Binding

The plasma protein binding of β-elemene in rat plasma was determined by equilibrium dialysis. The compound was highly bound with protein binding values of 97.7%±0.7% and 96.5%±0.4% at the concentration of 0.06 µg/ml and 0.1 µg/ml respectively, demonstrating that β-elemene bound extensively to rat plasma.

### Excretion

Less than 2% of the dose was recovered in bile, urine or feces as parent drug β-elemene, indicating the drug was extensively metabolized after i.v. administration.
Testis, Plasma, Muscle, GI, lung, Brain, Liver, Fat, Spleen, Kidney, Heart

Concentration (µg/g wet tissue)

Fig. 3. Tissue distribution of β-elemene after a single iv administration at the dosage of 75 mg / kg to rats. The data were the mean of 5 rats. The vertical indicate the SD point

Table 2. Tissue plasma ratio after a single i.v. administration to rats (Dose: 100 mg/kg)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>heart</th>
<th>kidney</th>
<th>spleen</th>
<th>fat</th>
<th>liver</th>
<th>lung</th>
<th>brain</th>
<th>GI</th>
<th>muscle</th>
<th>testis</th>
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</thead>
<tbody>
<tr>
<td>15min</td>
<td>5.86</td>
<td>4.74</td>
<td>3.50</td>
<td>3.11</td>
<td>2.81</td>
<td>2.22</td>
<td>2.48</td>
<td>1.47</td>
<td>1.23</td>
<td>0.50</td>
</tr>
<tr>
<td>180min</td>
<td>3.30</td>
<td>2.53</td>
<td>1.16</td>
<td>9.04</td>
<td>0.30</td>
<td>1.67</td>
<td>7.95</td>
<td>1.08</td>
<td>2.22</td>
<td>2.34</td>
</tr>
</tbody>
</table>

Identification of Metabolites in rat bile

A typical gas chromatogram of bile sample after β-elemene administration to rats is shown in Fig. 4. One unexpected metabolite peak (M-1) was observed. To better understand the elimination of β-elemene in rats, in vivo metabolites were determined. M-1 was isolated from rat bile and identified on the basis of GC/MS and NMR analyses. The GC-mass spectra fragmentation pattern of M-1 was similar to that of β-elemene authentic reference sample except that M-1 has extra molecular weight of 14 (Tab 3). Table 4 lists the acquired GC-MS spectra data of both M-1 and β-elemene standard. The 1H NMR spectrum of β-elemene (Fig. 5) contained signals for three methyl groups (δ =1.0006, δ =1.70799 and δ =1.74184), while M-1 showed two methyl groups (δ =1.02554, δ =1.70428) and one aldehyde group (δ =9.5301). The 13C NMR spectrum of M-1 (Fig 6) contained signals of aldehyde group (δ =194627).

Table 3. MS data of β-elemene and M1 in rat bile

<table>
<thead>
<tr>
<th>compound</th>
<th>m/z</th>
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<tbody>
<tr>
<td>β-elemene</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>81</td>
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<tr>
<td></td>
<td>189</td>
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<tr>
<td></td>
<td>204</td>
</tr>
<tr>
<td>M1</td>
<td>53</td>
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<td></td>
<td>67</td>
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<td></td>
<td>81</td>
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<td>218</td>
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</tbody>
</table>

Table 4. 13CNMR data (δ) of β-elemene and M1 in rat bile

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<tr>
<th>C</th>
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<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
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<tbody>
<tr>
<td>a</td>
<td>39.88</td>
<td>32.19</td>
<td>26.84</td>
<td>45.73</td>
<td>39.93</td>
<td>52.77</td>
<td>16.63</td>
<td>150.37</td>
<td>109.63</td>
<td>147.69</td>
<td>21.04</td>
<td>108.23</td>
<td>150.30</td>
<td>24.75</td>
<td>112.08</td>
</tr>
<tr>
<td>b</td>
<td>32.73</td>
<td>30.28</td>
<td>26.76</td>
<td>39.65</td>
<td>36.34</td>
<td>52.37</td>
<td>16.50</td>
<td>154.75</td>
<td>112.18</td>
<td>147.29</td>
<td>194.63</td>
<td>110.07</td>
<td>149.94</td>
<td>24.92</td>
<td>132.99</td>
</tr>
</tbody>
</table>

a: β-elemene standard; b: M1
Discussion

These studies were conducted to characterize the pharmacokinetics, distribution, metabolism, and excretion of β-elemene in male SD rats. The results from this study demonstrate that β-elemene distributed to most tissues and organs, and metabolized extensively before excretion. The AUC values increased fairly proportionally to the dose, indicating that the pharmacokinetics of β-elemene were linear. The half-life of elimination was relatively short, being approximately 1 hour. The pharmacokinetics parameter $V_{dss}$ values were between 1.9-2.6 liters / kg, indicating that β-elemene was highly distributed to tissues, and this was further confirmed by tissue distribution experiments. The drug distributed preferentially into well perfused organs such as liver and kidney at an early time point (15 min) and was also detectable in most of the tissues after i.v. administration at all time points (Fig 3 Table 2). Notably, brain / plasma concentration ratio of drug was 2.67, 8.57, and 7.68 at 15 min, 30 min, and 180 min after iv administration respectively, suggesting that β-elemene effectively crossed the blood-brain barrier and penetrated into the brain. The great lipophilicity of β-elemene allows a high uptake of the drug into the brain. Elemene has proven clinically to be significantly effective for the
treatment of malignant brain tumors by prolonging the high quality survival time of patients. The ability
of β-elemene to cross the blood-brain barrier with high brain penetration achieves and maintains the
high concentration observed in rats, which is in good agreement with its clinical utility for the treatment of
malignant brain tumors and of metastasis brain
tumor diseases. The accumulation of the drug in the
brain may contribute to the basis of its clinically
therapeutic uses. Likewise the high lipophilicity of
β-elemene may potentially facilitate its accumulation
in the fat deposits. Moreover, fat tissue / blood ratios
at all three time points (15 min, 30 min, and 180 min)
tested were 3.35, 4.15, and 8.74 respectively, clearly
suggesting a very high affinity of the compound for
the fat system, with the highest concentration in the
fat deposits after 3 h. However whether the deposit
of β-elemene in brain and fat leads to toxic effects or
not is unclear in the present investigation.

Very little unchanged β-elemene was excreted
via urine, feces, and bile. Excretion was rapid, with
0.61% of the drug being excreted in feces during 32
h. Also less than 0.01% of the administered
β-elemene was excreted in urine as unchanged
parent drug, and only 0.06% drug excreted from bile,
indicating that β-elemene was almost completely
metabolized in rats before elimination.

The major metabolite of β-elemene in rat bile
was isolated and identified. The GC / MS
characteristic fragmentation for β-elemene and its
metabolite allowed for the speculative M1 molecular
weight assignment as 218. Comparison of the NMR
proton resonances for the metabolites to those for
parent drug facilitated assignment of the substitution
position, that the methyl of No. 11 carbon
connecting with No. 10 carbon was oxidized to an
aldehyde. Infrared spectrometry and ultraviolet
spectrometry proved that the aldehyde existed in the
metabolite (data not shown). The structure of the M1
metabolite was 1-methyl-1-ethenyl-2-isopropenyl-4-
isopropenyl aldehydehexamethylene.

In summary, β-elemene was widely distributed
to most tissues and organs, rapidly excreted, and
metabolized extensively before excretion in male SD
rats. Its effectiveness in crossing the blood-brain
barrier holds promise for the treatment of malignant
brain tumors. Biotransformation of β-elemene exists
in rat and the metabolite in rat bile was identified.
The time-course and kinetic characteristics of the
metabolite formed in rat liver need further
investigation.

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  - Mechanism of drugs (including molecule, cells and gestalt levels)
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  - Clinical reports of new drugs on effectiveness and safety
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