Study of Target Pegylated Recombinant Mutant Human Granulocyte Colony Stimulating Factor

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Abstract Recombinant mutant human granulocyte colony stimulating factor\(^1\) mhmG-CSF\(^1\) was pegylated\(^1\) purified and characterized . rhG-CSF was mutated in position 141\(^1\) and cysteine was added in C-terminal . mhmG-CSF was pegylated by PEG-Mal 20000 and separated by ion-exchange chromatography\(^1\) gel filtration chromatography . Analysis of SDS-PAGE showed that the purity of the separated PEG-mhmG-CSF was greater than 95% . and in intro and in vivo bioactivity study showed that target modified PEG-mhmG-CSF kept full bioactivity which was better than traditional pegylation method\(^1\) and longer half-life was proved in mice .

Key words pegylation\(^1\) recombinant mutant human granulocyte colony stimulating factor\(^1\) purification\(^1\) characterization

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酰胺化的半胱氨酸的终产物可能是不同修饰位点的混合物,还可能有多义。我们构建了一种试剂均为国产分析纯或生化纯试剂。

现有临床应用的产品是利用活化剂、柱式质粒小提试剂盒、胶回收试剂盒、酶切反应、连接酶、电泳、染色、脱盐层析、浓缩胶为聚丙烯酰胺凝胶,电泳、扫描、分析。通过对其聚合酶、柱式质粒小提试剂盒、胶回收试剂盒、酶切反应、连接酶、电泳、染色、脱盐层析、浓缩胶为聚丙烯酰胺凝胶,电泳、扫描、分析。产品是利用活化剂、柱式质粒小提试剂盒、胶回收试剂盒、酶切反应、连接酶、电泳、染色、脱盐层析、浓缩胶为聚丙烯酰胺凝胶,电泳、扫描、分析。治疗方法包括在电泳中,以提高生物学活性,并在其结构中加入一个氨基,因此所获得的产物,具有很重要的临床和经济意义。我们构建了一种试剂均为国产分析纯或生化纯试剂。

目的基因的扩增。

1.2.1

1.2.2

1.3

1.3.1

1.3.2
1.3.3 RP-HPLC  
Vydec C18 150mm × 4.6mm I.D. A 1% B W/V. B (0-90) 0.1% B (0-30) 80% A 80% B 25% 0.8mL/min 30min B 280nm

1.3.4  Superdex 200 HR10/30 0.1mol/L. B 0.02mol/L. A 0.1mol/L. B 180 36 ~ 48h A50/A630

1.3.5 AutoFlex MALDI-TOF-MS  
mhG-CSF PEG-mhG-CSF

1.3.6 NFS60 G-CSF 96 50 μL/100μL rhG-CSF 50μL 50μL

1.3.7 18 ~ 22g SPF 1mg/kg rhG-CSF 0.083.0.25.0.5.124h 3 8124h 3 Human G-CSF DuoSet ELISA rhG-CSF PEG-mhG-CSF MicroCal Origin 3P87

2  

2.1 rmhG-CSF  hG-CSF  Ala Thr Tyr

Arg Ser Cys pET32a Nde I / BamH I  2 5.4kb 540bp 17 64 3 3 Resouse S

2.2 rhmG-CSF PEG-MAL20000 5 PEG-mhG-CSF 2rhG-CSF 50% 4

ATG GCA ACA AYA TCT TCT TCT CGG CAG ACG TTT TCT
M A P T Y R A S S L P Q S F L
CTG AAA TCT GAA CAG GCT TCT GCT AAA ATC CAG CSG GCT GCT
L K S L E Q V R I Q G D G A

... TAC CGT GGT CGT CAT CGT GCT CGT CCG TSG TAG...

... T Y V R L H A Q C P * *

1 1 mhG-CSF cDNA N 10 N 0 C 10 C 10 N 0 C 10 C 10

Fig. 1 cDNA and protein sequence of mhG-CSF

2 2 mhG-CSF  3DNA marker  EcoR I  + Hind II

3 3 mhG-CSF SDS-PAGE 4

Fig. 2 Identification of the gene of mhG-CSF detected by agarose gel electrophoresis

1) the vector pET32a was digested by BamH I 2) The recombinant expression plasmid pET32a-mhG-CSF was digested by Nde I / BamH I 3) DNA marker EcoRI  + Hind III

Fig. 3 Expression and purification of mhG-CSF detected by SDS-PAGE

1) DNA marker 2) Expression of mhG-CSF-Cys without and with IPTG-inducing 4) Equent from Resource S.

2.2 rmhG-CSF PEG-MAL20000 5 PEG-mhG-CSF 6 Resource S 7 Resource S
Fig. 4 The relative biological activity of rhG-CSF and rmhG-CSF.

Fig. 5 Pegylation of rmhG-CSF monitored by RP-HPLC. A] rmhG-CSF B] analyzed at 24h after pegylation.

Fig. 6 Chromatograph of PEG-rmhG-CSF on Resource S.

Fig. 7 Pegylation of rmhG-CSF and purification of PEG-rmhG-CSF detected by non-reduced SDS-PAGE. 1[Unligated] 2[purified rmhG-CSF] 3)mixture of pegylation reaction 4]Peak A 5]Peak B of Resource S.

Fig. 8 Analysis of rmhG-CSF and PEG-rmhG-CSF by MALDI-TOF-MS. A] rmhG-CSF B] PEG-rmhG-CSF.

2.3 PEG-rmhG-CSF

Neulasta [0.58 × 10^7/mg] C
PEG-rmhG-CSF
mhG-CSF [1.23 × 10^5 IU/mg] D
PEG-rmhG-CSF [1.25 × 10^8 IU/mg] E
PEG-rmhG-CSF F
PEG-rhG-CSF

Fig. 7 shows the relative biological activity of rhG-CSF, rmhG-CSF, and PEG-rmhG-CSF. The biological activity of rhG-CSF and rmhG-CSF is measured as 100 and 150, respectively.

The pegylation process is monitored by RP-HPLC. Figure 5 shows the chromatograph of rmhG-CSF before and after pegylation.

The chromatogram of PEG-rmhG-CSF on Resource S is shown in Figure 6.

PEG-rmhG-CSF and rmhG-CSF are analyzed by non-reduced SDS-PAGE in Figure 7. Peak A and Peak B are identified as Resource S.

Figure 8 shows the analysis of rmhG-CSF and PEG-rmhG-CSF by MALDI-TOF-MS. A is rmhG-CSF and B is PEG-rmhG-CSF.

The biological activity of rmhG-CSF and PEG-rmhG-CSF is shown in Figure 7. The biological activity of rmhG-CSF is 18.9 units, while that of PEG-rmhG-CSF is 40.715 units.

The half-life of rmhG-CSF in vivo is 14.6 hours, whereas that of PEG-rmhG-CSF is 100 hours.

PEG-rmhG-CSF is more stable than rmhG-CSF in the bloodstream.

The molecular weight of rmhG-CSF is measured by MALDI-TOF-MS, and in Figure 8, the molecular weight of PEG-rmhG-CSF is analyzed before and after pegylation.

The molecular weight of rmhG-CSF is 18.9 units, while that of PEG-rmhG-CSF is 40.715 units.

The half-life of PEG-rmhG-CSF in vivo is 100 hours, whereas that of rmhG-CSF is 14.6 hours.

PEG-rmhG-CSF is more stable than rmhG-CSF in the bloodstream.
1 PEG-rmhb-CSF

<table>
<thead>
<tr>
<th>Table 1</th>
<th>In vitro bioactivity of of rmhb-CSF-Cys before and after pegylation</th>
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<tbody>
<tr>
<td>Specific bioactivity</td>
<td>Relative bioactivity /%</td>
</tr>
<tr>
<td>rmhb-CSF</td>
<td>1.23</td>
</tr>
<tr>
<td>PEG-rmhb-CSF</td>
<td>1.25</td>
</tr>
<tr>
<td>PEG-rhG-CSF</td>
<td>0.58</td>
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2 rhG-CSF

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<tr>
<th>Table 2</th>
<th>Mean pharmacokinetic parameter values after single-dose subcutaneous administration of rhG-CSF and PEG-rmhb-CSF in mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Value</td>
</tr>
<tr>
<td>rhG-CSF</td>
<td>PEG-rmhb-CSF</td>
</tr>
<tr>
<td>Lag time/h</td>
<td>0.035 ± 0.005</td>
</tr>
<tr>
<td>T1/2/h</td>
<td>2.143 ± 0.025</td>
</tr>
<tr>
<td>T max/h</td>
<td>1.388 ± 0.235</td>
</tr>
<tr>
<td>C max [ng/mL]</td>
<td>1854.623 ± 253.125</td>
</tr>
<tr>
<td>AUC [ng h/mL]</td>
<td>8881.870 ± 524.57</td>
</tr>
<tr>
<td>CL/F [L/kg]</td>
<td>0.113 ± 0.023</td>
</tr>
<tr>
<td>V/F [L/kg]</td>
<td>0.349 ± 0.035</td>
</tr>
</tbody>
</table>

T1/2 = terminal half-life; T max = time of maximum concentration; C max = maximum concentration; AUC = area under the curve; CL/F = clearance over bioavailability; V/F = volume of distribution using the terminal phase.

3 rhG-CSF

Fig. 9 Concentration-time curve of PEG-rhG-CSF and rhG-CSF after SC in mice

REFERENCES