PEGylation for drug delivery to ischemic myocardium: Pharmacokinetics and cardiac distribution of poly(ethylene glycol)s in mice with normal and ischemic myocardium

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\textbf{Abstract}

PEGylation now plays an important role in drug delivery and is considered as the method of choice for improving the pharmacokinetics and stability of parenteral agents. However, its application in treating cardiac diseases is still limited. To guide the design of PEGylation for drug delivery to ischemic myocardium, the effects of the molecular weight of PEG and the myocardial ischemic conditions on PEG levels in plasma and myocardium were studied in this work following intravenous administration of fluorescein isothiocyanate-labeled 20- and 40-kDa mPEGs to mice with normal and ischemic myocardium. The results show that myocardial ischemia caused some consistent changes in pharmacokinetic parameters of mPEGs. Due to the enhanced permeability and retention (EPR) effect caused by ischemia, the distribution of 20- and 40-kDa mPEGs in ischemic hearts was approximately 1.47- and 1.92-fold higher than that in normal hearts, respectively. Under the same heart condition (either normal or ischemic), the cardiac AUC\textsubscript{0.5–24h} of the two mPEGs were comparable, although their plasma AUCs differed by nearly 4-fold; however, a smoother cardiac level-time profile was achieved by 40-kDa mPEG. This study addressed the relative importance of the EPR effect of ischemic zones and the molecular size of PEG in cardiac drug delivery, which is believed to be helpful for macromolecular drug design.

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1. Introduction

Ischemic heart disease, an imbalance between myocardial oxygen supply and demand, is one of the most common causes of death around the world, especially in middle and high-income countries. Approximately 7.25 million people died of this disease in 2008, accounting for 12.8% of the total death (WHO, 2011). Although there are many kinds of anti-ischemic drugs and adjuvants, including calcium channel blockers, \textbeta\textendash adrenergic blockers, and organic nitrates, whose mechanism of action is mainly via decreasing myocardial oxygen consumption, or others like anticoagulant, antiplatelet and thrombolytic medications which could increase coronary blood flow and oxygen supply, most of them lack in tissue specificity and have a relatively short elimination half-life and low accessibility to the sites of action within cells. These, together with a significantly reduced blood circulation in ischemic regions, always lead to a quite low drug distribution in the targets while causing adverse effects elsewhere (Sun and Lin, 2010). Therefore, for clinical applications, it is quite essential and meaningful to selectively accumulate drugs in ischemic myocardium.

As is well known, myocardial ischemia could cause many pathophysiological changes, including the enhanced permeability of endothelial cell membranes, the up-regulated expression of various cell adhesion molecules on the endothelium, the exposure of intracellular antigenic components, and a decreased pH within ischemic areas (Scott et al., 2008; Sun and Lin, 2010; Torchilin, 1995). Based on these changes, especially the enhanced permeability and retention (EPR) effect caused by ischemia (Lukyanov et al., 2004; Palmer et al., 1984; Rodriguez et al., 2005), passive targeting, active targeting, or systems responding to environmental pathologic alterations could be achieved in different ways. Presently, there are two main strategies to target drugs to the ischemic myocardium. One involves physical entrapment of bioactive substances in natural or artificial structures including liposomes, micelles, nanoparticles, microbubbles, cell ghosts, etc. (Arayne et al., 2007; Mayer and Bekeredjian, 2008; Torchilin, 1995). The other comprises chemical modification of therapeutic agents through covalent linkage to hydrophilic polymers like poly(ethylene glycol)
2.1. Materials and animals

with normal and ischemic myocardium.

PEGs to mice dial ischemic conditions on PEG levels in plasma and myocardium

the effects of the molecular weight of PEG and the myocar-
tance of the EPR effect in ischemic zones and the molecular size of

ischemic myocardium, in particular, to address the relative impor-

knowledge, no report on the distribution of PEGs in ischemic

myocardium.

PEGs might be
greatly improved, thus resulting in enhancement of therapeutic
efficacy and reduction in side effects. This, however, closely related with
the molecular size, structure, and charges of PEG or its deriv-
atives (Caliceti and Veronese, 2003), especially when the molecular
weight of the drug is low. Though distribution and tissue uptake of
PEG with different molecular weights had already been studied before
in normal mice (Yamaoka et al., 1994), there is, to our
knowledge, no report on the distribution of PEGs in ischemic
myocardium.

In order to guide the design of PEGylation for drug delivery to
ischemic myocardium, in particular, to address the relative import-
ance of the EPR effect in ischemic zones and the molecular size of
PEGs, the effects of the molecular weight of PEG and the myocar-
dial ischemic conditions on PEG levels in plasma and myocardium
were studied in the present work following intravenous adminis-
tration of fluorescein isothiocyanate (FITC)-labeled mPEGs to mice
with normal and ischemic myocardium.

2. Materials and methods

2.1. Materials and animals

Linear amino-terminated poly(ethylene glycol) methyl ether
(mPEG-NH$_2$) hydrochlorides with molecular weights of 20 and
40 kDa were purchased from Jenkem Technology Co., Ltd. (Beijing,
China). Fluorescein isothiocyanate (FITC) was purchased from Sigma
(St. Louis, MO, USA). Extra dry dimethyl sulfoxide (DMSO) was
purchased from Acros Organics (Geel, Belgium). Superdry pyridine
was purchased from J&K Scientific Ltd. (Beijing, China). All the
other chemicals were of reagent grade and purchased from com-
mercial sources.

One hundred and thirty male KM mice, weighing an average of
18–22 g, were supplied by the Lab Animal Center of Shanghai Uni-
versity of Traditional Chinese Medicine. They were kept in an envi-
ronmentally controlled breeding room for four days before starting
the experiments and fed with standard laboratory food and given
water ad libitum. The Animal Ethical Experimentation Committee of
Shanghai University of Traditional Chinese Medicine, according to
the requirements of the National Act on the Use of Experimental
Animals (PR China), approved all procedures of the animal
experiments.

2.2. FITC labeling of mPEG-NH$_2$ and characterization

In order to determine the levels of mPEG-NH$_2$ in plasma and
cardiac tissues after administration, they were pre-labeled with
FITC according to the method previously established (Lin et al.,
2010). In brief, 1 g of mPEG-NH$_2$ reacted with 0.1 g of FITC under
the catalysis of 20 mg of dibutyltin dilaurate in DMSO (10 ml
containing a few drops of pyridine for 30 min at 95°C. After reac-
tion, the product was precipitated by nine volumes of a cold
diethyl ether/ethanol (3:1, v/v) mixture and then filtered. The final
product was washed with the aforementioned precipitation re-
agent at least nine times till free FITC and the other excess reagents
were fully removed, and finally dried in vacuo. The amounts of FITC
conjugated to mPEG-NH$_2$s were determined by Hitachi F-4500
fluorescence spectrophotometer (Tokyo, Japan) and were calcu-
lated in terms of their fluorescence intensity.

FITC-labeled mPEG-NH$_2$s were characterized by high-performa-
gel permeation chromatography (HPGPC) on a Waters li-
quid chromatographic instrument equipped with a Shodex
OHpak SB-803 HQ gel-filtration column (300 x 8.0 mm) (Tokyo, Ja-
pan) and a Waters 2414 refractive index detector (Milford, USA).
The eluent of the system was 0.1 M phosphate buffer (pH 7.4)
and was delivered at a flow rate of 0.5 ml/min. The poly(ethylene
glycol) standards for GPC with weight-average molecular weight
of 0.232, 2.01, 6.55, 17.9, 42.7 and 118.0 kDa (Fluka, Buchs, Ger-
many) were used to calibrate the system. In addition, FITC-labeled
mPEG-NH$_2$s were determined by HPGPC with a fluorescence detec-
tor to verify the conjugation of FITC with mPEG-NH$_2$s.

2.3. Preparation of standard and quality control samples

Stock solutions of FITC-labeled 20- and 40-kDa mPEG-NH$_2$ in
distilled water at a concentration of 3.6 and 8.0 mg/ml, respec-
tively, were prepared. A series of standard solutions were then ob-
tained by further dilution of the two stock solutions with distilled
water, through which the concentrations were in the range of
0.05–2.4 and 0.05–4 mg/ml, respectively.

To prepare the standard calibration samples, an aliquot of 10 µl
of each standard solution placed in a 1.5 ml centrifuge tube was
blown dry with nitrogen gas followed by the addition of 100 µl
blank plasma or heart homogenates. After being thoroughly
homogenized by vortexing, the mixture was then treated accord-
ing to the sample pretreatment procedure described below. The fi-
nal standard plasma concentrations of 20- and 40-kDa mPEG-NH$_2$
were 10–360 and 50–800 µg/ml, respectively, while the final stan-
dard heart concentrations for the two mPEG-NH$_2$s were 5–120 and
5–200 µg/ml, respectively. The quality control (QC) samples used
in the method validation were prepared in the same way as the
standard calibration samples.

2.4. Sample pretreatment procedure and analytical conditions

For the pretreatment of heart homogenates, 21.5 µl of 1 M per-
chloric acid was added to a 50 µl of supernatant obtained from
100 µl portion of each centrifuged homogenate. The mixture was
vortexed and left to stand for 2 h before centrifuged at
10,000 rpm for 1 min to precipitate the denatured proteins. The
supernatant was then transferred to a new tube and neutralized
by the addition of 16 µl of 1 M NaOH. After centrifugation at
10,000 rpm for 1 min again, the supernatant was assayed by the
method described below. While for the pretreatment of plasma
samples, plasma proteins were precipitated by adding 40 µl of
1 M perchloric acid to a 100 µl portion of each sample. The super-
natant obtained was then neutralized by 30 µl of 1 M NaOH. With
another centrifugation, the supernatant was analyzed by HPLC in
the same way.

The assay system consisted of an Agilent 1200 series HPLC with
a fluorescence detector set at $\lambda_{ex}$ 495 nm and $\lambda_{em}$ 515 nm. Samples
were separated by HPGPC using a Shodex OHpak SB-803 HQ col-
umn. The eluent was 0.1 M phosphate buffer (pH 7.4), delivered at
a flow rate of 0.5 ml/min. The chromatographic procedures
were performed at 30°C.
2.5. Tissue distribution study

Mice with acute myocardial ischemia induced by isoprenaline (20 mg/kg daily by subcutaneous injection for four consecutive days), and normal mice, were given a single intravenous dose of the FITC-labeled mPEG-NH₂ via the tail vein. MPEG-NH₂s with molecular weights of 20 and 40 kDa were administered at the same molar dose of 6.80 µmol/kg. Then mice were sacrificed at the specified time points of 0.5, 1, 4, 12, and 24 h postdose. Blood samples were collected into tubes containing heparin and immediately centrifuged at 3000 rpm for 10 min. Meanwhile, hearts were harvested, rinsed three times with saline solution to wash away the residual blood, blotted on filter paper, and then homogenized by a 3-fold volume (m/v) of normal saline. The separated plasma and heart homogenates were stored at −20 °C until assay.

2.6. Data analysis

Data were expressed as means ± SD. Statistical analyses were assessed using Student’s t-test. Statistically significant differences were indicated by P values of <0.05. The DAS 2.0 pharmacokinetic software (Chinese Pharmacology Society) was used to calculate major pharmacokinetic parameters such as AUC (area under the curve), MRT (mean residence time), \( V_t \) (volume of distribution), and CL (systemic clearance) by non-compartmental analysis.

3. Results

3.1. Characterization of FITC-labeled mPEG-NH₂s

The degree of FITC substitution is approximately 1.10 and 0.94 mol FITC per mol 20-kDa and 40-kDa mPEG-NH₂, respectively. The results of HPGPC showed that FITC-labeled mPEG-NH₂s used in this study were highly homogeneous, with polydispersity index approaching 1.0 (Table 1). After FITC labeling, no remarkable differences in their peak shape and peak height were observed. However, their elution positions were both a bit postponed, which is believed to be due to their decreased hydrodynamic volume after conjugating with a hydrophobic group like FITC. By HPGPC-FLD assay, FITC-labeled mPEG-NH₂s could be detected easily while the unlabeled ones and free FITC had no peaks under the same condition, thus verified the conjugation of FITC with mPEG-NH₂ (Fig. 1). In addition, they were also believed to be stable in the whole process since no degradation products were ever detected.

3.2. Method validation

The degree of interference by the other substances was assessed by the inspection of chromatograms derived from processed blank and mouse samples. It was indicated that FITC-labeled mPEG-NH₂s were well separated and no interferences were detected from endogenous substances and metabolites (Fig. 1). The typical retention times for FITC-labeled 20- and 40-kDa mPEG-NH₂s were approximately 15.4 and 13.9 min, respectively.

Standard curves of the peak height (\( Y \)) to the concentration (\( C \)) were established using the 1/C or 1 weighted linear least squares regression model. Linearity was evident over the concentration range studied in plasma and heart tissues, with correlation coefficients larger than 0.998 (Table 2). The detection limit (DL) and quantification limit (QL) of the method calculated from the signal-to-noise ratio (DL and QL correspond to the 3- and 10-fold of the noise level, respectively) were approximately 0.22–1.19 µg/ml and 0.74–3.96 µg/ml, respectively (Table 2), indicating that the method was sensitive.

The precision and accuracy of the method were examined by adding known amounts of reference standards to blank mouse plasma and heart tissues. The results showed that the method was accurate and precise with the relative recoveries of FITC-labeled mPEG-NH₂s ranging from 90% to 115% and the within- and inter-batch R.S.D. values not more than 12.3% except that the inter-batch R.S.D. value of 20-kDa mPEG-NH₂ was less than 20% (Table 3). The absolute recoveries of mPEG-NH₂s were tested at three QC levels by comparing the peak height of pretreated mouse samples with those obtained by direct injection of standard solutions at the same concentration, which were found to be between 55.2% and 95.3% (Table 3).

The stability of FITC-labeled mPEG-NH₂s in biosamples was investigated under a variety of storage and process conditions including being kept at room temperature for 12 h, in a refrigerator (3–5 °C) for 24 h, or undergoing two freeze–thaw cycles. By comparing the results of QC samples analyzed before and after being exposed to the conditions for stability assessment, ratios between
comparison samples and stability samples were within 85–115% or 80–120%.

3.3. Plasma levels of FITC-labeled mPEG-NH₂s

Pharmacokinetic properties of mPEG-NH₂s were evaluated in both myocardial ischemic and normal mice following i.v. administration. Blood plasma concentration–time profiles are shown in Fig. 2 with detailed data available in Table 4. All the main pharmacokinetic parameters calculated by non-compartmental model analysis are summarized in Table 5. It is apparent that both mPEGs displayed a multi-compartmental pharmacokinetic behavior. As the molecular weight of mPEG increased from 20 to 40 kDa, the elimination half-lives and AUC values were multiplied several times. This coincided with a 4- to 5-fold higher systemic clearance of 20-kDa mPEG than 40-kDa mPEG, which indicated an abrupt change in renal excretion around the glomerular filtration threshold for PEG (C24 kDa) (Greenwald et al., 1996; Yamaoka et al., 1994). As a whole, approximately 25% of the 40-kDa mPEG of the injected dose was retained at 24 h postdose, whereas only about 2% of the 20-kDa mPEG could be detected after 24 h.

Although insignificant differences of most mPEG plasma levels in mice with normal and ischemic myocardium suggest that pathological conditions caused by myocardial ischemia had limited impact on PEG concentrations in systemic circulation (Table 4), the calculated pharmacokinetic parameters do show some consistent changes in mice with ischemic myocardium (Table 5), such as the prolongation of t1/2 and MRT, the decrease of CL, and the increase in V, regardless of the molecular mass of mPEGs. The former two changes were considered to be caused by the decreased cardiac output due to the ischemia and the last might involve the increased permeation of capillaries in ischemic myocardium and reduced blood flow rate. In a previous report, He et al. (2004) also found that the injuries caused by ischemia–reperfusion could significantly increase AUC, decrease CL, and prolong the terminal half-life of paoniflorin in rats.

3.4. Cardiac distribution of FITC-labeled mPEG-NH₂s

The molar concentrations of 20- and 40-kDa mPEGs in cardiac tissues from normal and myocardial ischemic mice are listed in Table 4, which showed that, except for the 24-h time point, both mPEGs had a significantly increased number of molecules in ischemic hearts at each time point postdose (P < 0.05 or 0.01) when compared to normal hearts. In terms of AUC0.5–24h, calculated by the trapezoidal rule, the distribution of 20- and 40-kDa mPEGs in ischemic hearts was approximately 1.47- and 1.92-fold higher than that in normal hearts, respectively, which indicated that both of their hydrodynamic volumes were small enough to ensure that the EPR effect caused by ischemia (Lukyanov et al., 2004; Palmer

### Table 2

<table>
<thead>
<tr>
<th>mPEG-NH₂ (kDa)</th>
<th>Biosample</th>
<th>Standard curves (n = 5–6)</th>
<th>Linear ranges (mg/ml)</th>
<th>DL (µg/ml)</th>
<th>QL (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Plasma</td>
<td>Y = 420.826C-1.210</td>
<td>0.01–0.36</td>
<td>0.22</td>
<td>0.74</td>
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<tr>
<td>20</td>
<td>Myocardium</td>
<td>Y = 383.351C–2.557</td>
<td>0.005–0.12</td>
<td>1.19</td>
<td>3.96</td>
</tr>
<tr>
<td>40</td>
<td>Plasma</td>
<td>Y = 192.932C–0.543</td>
<td>0.05–0.80</td>
<td>0.36</td>
<td>1.20</td>
</tr>
<tr>
<td>40</td>
<td>Myocardium</td>
<td>Y = 169.347C–0.594</td>
<td>0.005–0.20</td>
<td>1.18</td>
<td>3.93</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>mPEG-NH₂ (kDa)</th>
<th>Biosample</th>
<th>Added conc. (mg/ml)</th>
<th>Recovery (%)</th>
<th>Accuracy (%)</th>
<th>Precision R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Within-batch</td>
<td>Inter-batch</td>
<td></td>
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<tr>
<td>20</td>
<td>Plasma</td>
<td>0.30</td>
<td>69.6 ± 3.7</td>
<td>107 ± 5.2</td>
<td>4.91 18.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.20</td>
<td>55.2 ± 2.8</td>
<td>90.2 ± 4.4</td>
<td>4.90 7.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.60</td>
<td>58.3 ± 4.4</td>
<td>91.8 ± 6.9</td>
<td>7.51 6.53</td>
</tr>
<tr>
<td>20</td>
<td>Myocardium</td>
<td>0.10</td>
<td>88.8 ± 9.1</td>
<td>112 ± 4.6</td>
<td>4.15 5.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.40</td>
<td>64.9 ± 1.7</td>
<td>101 ± 2.2</td>
<td>2.16 5.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.20</td>
<td>69.7 ± 4.8</td>
<td>113 ± 7.5</td>
<td>6.62 5.93</td>
</tr>
<tr>
<td>40</td>
<td>Plasma</td>
<td>0.50</td>
<td>91.6 ± 2.4</td>
<td>96.0 ± 2.4</td>
<td>2.48 9.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.00</td>
<td>86.8 ± 4.7</td>
<td>95.6 ± 5.1</td>
<td>5.33 7.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.00</td>
<td>85.7 ± 4.2</td>
<td>91.0 ± 4.5</td>
<td>4.95 3.65</td>
</tr>
<tr>
<td>40</td>
<td>Myocardium</td>
<td>0.05</td>
<td>71.0 ± 12.1</td>
<td>115 ± 5.2</td>
<td>4.57 12.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20</td>
<td>81.6 ± 7.5</td>
<td>96.9 ± 3.3</td>
<td>3.38 4.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.00</td>
<td>95.3 ± 4.3</td>
<td>109 ± 4.6</td>
<td>4.24 4.58</td>
</tr>
</tbody>
</table>

### Fig. 2

Plasma levels of FITC-labeled 20-kDa mPEG-NH₂ (up- and down-triangles) and 40-kDa mPEG-NH₂ (squares and circles) versus time profiles following intravenous administration at a dose of 6.80 µmol/kg to normal mice (green down-triangles and circles) and mice with myocardial ischemia (red up-triangles and squares). Each data point represents the mean ± SD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

et al., 1984; Rodriguez et al., 2005) could overwhelm the negative effect of the decreased blood flow on distribution. In addition, the cardiac level-time profiles for 20- and 40-kDa mPEGs also showed some differences (Fig. 3). Compared to 40-kDa mPEG, the 20-kDa mPEG exhibited faster distribution into and elimination from cardiac tissues, whose level in cardiac tissues was almost twice as much as that of 40-kDa mPEG at 0.5 h post-dose, but just 65–90% that of 40-kDa mPEG at 24 h postdose. In comparison with their plasma levels, the levels of both mPEGs in cardiac tissues fluctuated less with time, but with different extent. For 20-kDa mPEG, its plasma level decreased by approximately 44 times from 0.5 to 24 h postdose; meanwhile, its cardiac level only decreased by about 5.8 times. During the same period of time, the decreased multiples for the levels of 40-kDa mPEG in plasma and cardiac tissues were, however, just somewhat different (3.6 and 2.4, respectively). These results indicated that the difference between the velocities of cardiac distribution and renal excretion was much bigger for 20-kDa mPEG than for 40-kDa mPEG. In summary, under the same heart condition (either normal or ischemic), the cardiac AUC_{0.5–24h} of 20- and 40-kDa mPEGs were comparable after i.v. administration at the same molar dose, although nearly a 4-fold difference in plasma AUC was observed. However, a smoother cardiac level-time profile was achieved by 40-kDa mPEG as compared with 20-kDa mPEG.

4. Discussion

A variety of nanosized insoluble drug delivery systems (e.g., nanoparticles, liposomes and micelles) and soluble polymeric drugs are emerging as a class of therapeutics for cancer with enhanced efficacy, while simultaneously reducing side effects, owing to properties such as selective distribution in tumors by the EPR effect alone or in combination with certain active targeting mechanism(s) (Davis et al., 2008). By contrast, only limited advances in the field of targeted drug delivery to diseased cardiac tissues are achieved and have their roots in targeted drug delivery

Table 4

Pharmacokinetic parameters following intravenous administration of FITC-labeled 20- and 40-kDa mPEG-NH₂ in mice with normal and ischemic myocardium (n = 5–6).

<table>
<thead>
<tr>
<th>PEG (kDa)</th>
<th>Biosample</th>
<th>Group</th>
<th>Concentration (µmol/l)</th>
<th>AUC_{0.5–24h} (mmol h/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 h</td>
<td>1 h</td>
<td>4 h</td>
</tr>
<tr>
<td>20</td>
<td>Myocardium</td>
<td>Normal</td>
<td>3.06 ± 0.887</td>
<td>3.01 ± 1.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemic</td>
<td>4.33 ± 0.352</td>
<td>4.56 ± 0.805</td>
</tr>
<tr>
<td>40</td>
<td>Myocardium</td>
<td>Normal</td>
<td>1.59 ± 0.446</td>
<td>1.00 ± 0.265</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemic</td>
<td>2.37 ± 0.319</td>
<td>1.75 ± 0.630</td>
</tr>
<tr>
<td>20</td>
<td>Plasma</td>
<td>Normal</td>
<td>118 ± 7.22</td>
<td>80.8 ± 10.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemic</td>
<td>115 ± 10.2</td>
<td>73.0 ± 2.24</td>
</tr>
<tr>
<td>40</td>
<td>Plasma</td>
<td>Normal</td>
<td>125 ± 8.10</td>
<td>113 ± 10.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ischemic</td>
<td>139 ± 10.7²</td>
<td>121 ± 8.97</td>
</tr>
</tbody>
</table>

* *P* < 0.05 and ^b^ *P* < 0.01, compared with hearts taken from normal mice.
^c^ *P* < 0.05 and ^d^ *P* < 0.01, compared with plasma taken from normal mice.

Fig. 3. Cardiac levels of FITC-labeled 20-kDa mPEG-NH₂ (A) and 40-kDa mPEG-NH₂ (B) versus time profiles following intravenous administration at a dose of 6.80 µmol/kg to normal mice (green circles) and mice with myocardial ischemia (red squares). Each data point represents the mean ± SD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
to tumors (Scott et al., 2008; Sun and Lin, 2010; Torchilin, 1995). In particular, there are few reports on using PEGylation as a strategy for cardiac targeting, although it now plays an important role in drug delivery and is considered as the method of choice for improving the pharmacokinetics and stability of parenteral agents (Kang et al., 2009; Pasut and Veronese, 2009). In one of our recent studies, it was found that the distribution of a bioactive polysaccharide in ischemic mouse hearts was increased by approximately 30-fold after PEGylation (Lin et al., 2011), indicating PEG modification might also be promising in treating heart diseases. Therefore, in this work, we further investigated the pharmacokinetics and cardiac distribution of two commonly used PEGylation agents in mice with normal and ischemic myocardium. The results obtained were believed to be conducive to such kind of PEGylation design and the estimation of targeting efficacy that could be achieved by this strategy.

In terms of those PEG modifiers commonly used in researches and on the market as well as 30 kDa being the cut-off molecular weight of glomerular membrane for PEGs’ permeation (Greenwald et al., 1996; Yamaoka et al., 1994), PEGs with molecular weight of 20 and 40 kDa were finally adopted in this work. PEGs with molecular mass above 40 kDa are considered to be unsuitable for PEGylation due to its limited further increase in elimination half-life and, more importantly, its increased possibility of accumulation in the body (Kang et al., 2009; Pasut and Veronese, 2009). PEG, like all the other synthetic polymers, is polydisperse. This leads to a population of PEGylated drug conjugates, which might have different biological properties, mainly in plasma half-life and tissue distribution. Therefore, the trend of development is to use PEGs with higher homogeneity for PEGylation, especially when dealing with low molecular-weight drugs, where the conjugates’ characteristics are more relevant to the mass of linked PEG. In this work, commercially available linear amino-terminated methoxy-PEGs were used due to their narrow polydispersity and high efficiency of fluorescence derivatization with FITC. The latter made possible the determination of FITC-labeled mPEG-NH₂ in both mouse plasma and hearts.

Apart from size, the surface charge of particles has also been found to play a certain role in drug delivery to diseased sites with high vascular permeability. For example, positively charged and neutral particles were definitely confirmed capable of preferential accumulation in the infarction zone as compared with negatively charged ones (Caride and Zaret, 1977; Caride et al., 1984; Palmer et al., 1984), which indicated the formation or/and exposure of negatively charged species in the infarction zone. However, the EPR effect, which was shown by many tumor studies to be size-dependent (Maeda, 2001; Maeda et al., 2001; Yuan et al., 1995), was also deemed as the primary mechanism of the spontaneous accumulation of the negatively charged PEG-PE micelles (7–20 nm) (Lukyanov et al., 2004) and long-circulating liposomes (120–150 nm) (Torchilin et al., 1996) in the infarcted zone. Therefore, the accumulation of PEGylated drugs, whose size is usually several to tens of nm and whose charge is always low if any, in ischemic myocardium should also be size-dependent.

Generally, coronary artery ligation and isoproterenol injection are commonly used methods to prepare animal models with myocardial ischemia for the in vivo activity evaluation of drugs (Bharti et al., 2010; Wexler, 1978). Both are effective to induce myocardial ischemia or infarction. The differences are that the former causes a larger infarct zone mainly in the left ventricle with most myocardial cells completely infarcted, whereas the latter leads to smaller and scattered infarct zones in the whole heart with some survived myocardial cells seen within infarcted areas (Liu et al., 2010). These two methods have their own advantages and characteristics, to choose which might be based on the following requirements such as ease of experimental manipulation, reproducibility of model, mortality of animals, cost, and availability. In the study, the ability of PEGs to localize in ischemic myocardium was studied in mice treated with isoproterenol. The successful model construction was confirmed by several pieces of evidence, including (i) a direct observation of accelerated heartbeat and decreased spontaneous motor activity immediately after the subcutaneous injection of isoproterenol and (ii) a collapsed heart with pale apex when it was taken out.

In this work, 20- and 40-kDa mPEGs were administered at the same molar dose rather than mass. The reasons for such a design include that (i) for PEGylated drugs, their molecular number rather than mass in the targets is more relevant to their bioactivity, especially when the molecular weight of native drugs is relatively low, and (ii) the in vivo diffusion behaviors (e.g., distribution and excretion) of different molecular-weight PEGs could be better compared based on the same initial molecular number. In general, there are three main factors that would affect drug distribution in the ischemic zone, that is, the tissue perfusion flow rate, the elimination half-life of drug, and the vascular permeability. The first two decide the drug amount and duration on distribution while the last affects the efficacy of distribution. Myocardial ischemia causes a decrease in blood flow (the ratio of blood flow in the normal to ischemic myocardial region was reported to be approximately 10:1 (Mueller et al., 1981)), which, on the one hand, reduces the total amount of PEG supplied to the ischemic region, causing an adverse effect on distribution, and, on the other hand, weakens the shearing force above the luminal surface of the blood vessel endothelium and thus prolongs the retention time of PEG for diffusing through the endothelium, causing increased chances for distribution. In order to better figure out the correlation between the molecular weight of PEG and its cardiac distribution, the results obtained from a 20-kDa mPEG monomodified polysaccharide named 1.04P20k-R with apparent molecular weight of 23.8 kDa (Lin et al., 2011) were analyzed together with the results obtained here. As shown in Fig. 4, with the molecular weight of the PEG-based macromolecules extending from ~20 to ~40 kDa, their MRT increased sharply.
which is reasonable as the molecular-weight range studied is just around the cut-off value of glomerular filtration for PEGs and is favorable to their cardiac distribution; while on the other hand, their decreased distribution tendency (i.e., the ratio of cardiac AUC0.5–24h to normal cardiac AUC0.5–24h) caused by the EPR effect was enhanced with the increase in molecular weight (Fig. 5). What needs to be mentioned is that the true values of targeting efficacy should be considered from a variety of perspectives including the characteristics of the disease, improvements in pharmacokinetics, the retention of pharmacodynamics, the reduction in toxicities, etc. As for 20-kDa mPEG, it has a quicker distribution to hearts and weaker shielding effects that are favorable for realizing quicker and better therapeutic efficacy of drugs, while for 40-kDa mPEG, it has a smoother cardiac level-time profile and higher targeting efficacy that are beneficial especially for drugs with narrow therapeutic window or severe cardiac toxicity. Taking into consideration the gravity of the ischemic event, 20-kDa mPEG might be more suitable to conjugate with drugs treating acute myocardial ischemia, since the quicker the biodistribution of the drug to the heart the better, thus a smoother cardiac level profile might be inappropriate under such a circumstance.

In addition, it was found that the passive targeting efficacy of PEG (calculated as the ratio of ischemic cardiac AUC0.5–24h to normal cardiac AUC0.5–24h) caused by the EPR effect was enhanced with the increase in molecular weight (Fig. 5). What needs to be mentioned is that the true values of targeting efficacy should be higher than those reported here since PEG levels in ischemic myocardium were measured by the treatment of the whole ischemic heart that is composed of not only ischemic myocardium but also normal and totally infarcted areas. This might not change the above-mentioned relationship a lot since the degree of underestimation for all the macromolecules compared was believed to be similar. Besides, the blood remained in the heart samples collected, if any, would also affect the results to some extent. However, because the blood flow is much more abundant in normal hearts than in ischemic ones, there should be more residual blood, if any, in the former. This, again, would lead to an underestimation on targeting efficacy with a similar degree for the macromolecules compared. Finally, unlike insoluble carriers, the linear and flexible structure of the PEG chain can help the polymer to cross the endothelium by a ‘snake-like’ movement, which would allow PEGs and PEGylated drugs whose apparent molecular weight appears to surpass the pore size of the endothelium to pass through, although at lower rates.

5. Conclusion

Myocardial ischemia made only limited impact on the pharmacokinetics of both 20- and 40-kDa mPEGs; however, it significantly enhanced their cardiac distribution due to the EPR effect caused by ischemia. Moreover, a comparative cardiac AUC was achieved by 20- and 40-kDa mPEGs in both normal and ischemic mice after i.v. administration at the same molar dose, although their plasma AUCs differed by nearly 4-fold. By comparison, 20-kDa mPEG exhibited a quicker distribution to and elimination from myocardia while 40-kDa mPEG achieved a smoother cardiac level-time profile and a higher targeting efficacy. As a whole, the results of this study provide some useful information on the design of PEGylation for drug delivery to ischemic myocardium, which should be decided case by case according to a variety of perspectives, including the characteristics of the disease, the improvement in pharmacokinetics, the retention of pharmacodynamics, the reduction in toxicities, etc.

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References


Fig. 5. The cardiac targeting efficacy (AUGischemic heart/AUGnormal heart) of 20- and 40-kDa mPEGs together with a 20-kDa mPEG monomodified polysaccharide, 1,042;R, (Lin et al., 2011) in mice.

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