Improved stability and tumor targeting of 5-fluorouracil by conjugation with hyaluronan

Zhikui Dong, Wenyi Zheng, Zaiyang Xu, Zongning Yin

Key Laboratory of Drug Targeting and Drug Delivery Systems, West China School of Pharmacy, Chengdu, Sichuan Province, 610041, China
yzn@scu.edu.cn

ABSTRACT SUMMARY
A novel hyaluronan-5-fluorouracil (HA-5-Fu) polymeric prodrug was designed and synthesized, employing adipic acid dihydrazide and succinic anhydride as linkage materials. HA-5-Fu to various solutions and carcinoma cell lines demonstrated enhanced stability and cytotoxicity compared to free 5-Fu, and the in vivo study further verified improved tumor targeting.

INTRODUCTION
5-Fu was commonly used as an anti-proliferation drug to treat carcinoma clinically by inhibiting DNA biosynthesis. It was proposed that incorporation of 5-Fu into particles or derivation into prodrugs to decrease drug metabolism confined by short maintenance time after intravenous injection as recommended. Besides, polymeric prodrug investigated in this work exhibited several other merits as a macromolecule, such as optimized permeability to gain better use of enhanced permeation and retention effect (EPR), convenient modification with functional molecules and sustained-release feature because of decelerated hydrolysis and enzymolysis due to stereospecific blockade.

HA is a linear, negatively charged polysaccharide consisting of repetitive disaccharides and serves multifunction as pericellular and extracellular matrix, notably as a main ligand of hyaladherin overexpressed on carcinoma cell surface. Herein, HA was used as both carrier material and targeting molecule aiming to increase stability and tumor targeting of 5-Fu. Meanwhile, time-consuming and labor intensive synthesis processes were avoided resulting from exempted modification of targeting ligands.

EXPERIMENTAL METHODS
HA-5-Fu was synthesized by three main procedures: activation of 5-Fu by esterification, derivation of HA (101 kDa) with adipic acid dihydrazide and final conjugation followed by multistep purification. The molecular weight and structure of HA-5-Fu were confirmed by Gel Permeation Chromatography and $^1$H-NMR, IR. The drug loading was determined after vigorous alkaline hydrolysis.

The in vitro hydrolysis and enzymolysis stability were evaluated by incubation HA-5-Fu with a series of PBS, hyaluronidase (HAsE) solution and plasma separately. Samples were withdrawn periodically and centrifuged, furnishing supernatant of released 5-Fu. The anti-proliferation activity of HA-5-Fu was estimated by MTT assay, considering the effects of incremental drug concentrations, incubation times and various cell lines.

Lastly, the in vivo pharmacokinetics study approved by Animal Ethical Experimentation Committee of Sichuan University was conducted. The drug content both in plasma and tumor of different times was determined after injection into tumor-bearing mice via the tail vein and subsequent biological sample extraction processes.

5-Fu level was determined by HPLC system equipped with Kromasil C18-ODS column throughout the study. The detector was set at 266 nm corresponding to the maximum absorbance for 5-Fu.

RESULTS AND DISCUSSION
The HA-5-Fu (MW 136 kDa) with drug loading of 87.674 mg/g was eventually obtained (Yield 17.8%) as illustrated in Figure 1. $^1$H-NMR (400 MHz, D$_2$O): δ (ppm): 8.023~8.056
over 5-Fu depended on drug concentration, incubation time and cell type, conclusively. It was worth mentioning that the susceptibility of different cell types to HA-5-Fu was as listed below: A2780> HepG2> Hela.

After injection into the mice, HA-5-Fu displayed longer maintenance time, lower clearance and higher bioavailability than free 5-Fu both in circulation and tumor as listed in Table 1.

Table 1. Pharmacokinetic parameters of 5-Fu and HA-5-Fu in plasma and tumor

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<thead>
<tr>
<th>Parameter</th>
<th>Plasma</th>
<th>Tumor</th>
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<tbody>
<tr>
<td></td>
<td>5-Fu</td>
<td>HA-5-Fu</td>
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<tr>
<td>T1/2 (min)</td>
<td>44</td>
<td>435</td>
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<tr>
<td>CL (min)</td>
<td>0.035</td>
<td>0.001</td>
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<tr>
<td>AUC0-t (mg/L*min)</td>
<td>530</td>
<td>12625</td>
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CONCLUSION
A novel conjugate HA-5-Fu was successfully synthesized and systematically evaluated in vitro and vivo. The consistent results showed enhanced stability and improved tumor targeting, predicting HA-5-Fu as a promising antitumor drug candidate that warrant further investigation.

REFERENCES

ACKNOWLEDGEMENTS
Financial support was provided by National Natural Science Foundation of China (NO. 30973659).