Potent inhibition of Zika virus replication by curcumin - poly(sodium 4-styrenesulfonate) conjugates

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Introduction

In recent years, the Zika virus (ZIKV) has emerged from a neglected flavivirus to a health-threating pathogen that causes malformations and microcephaly in neonates as well as neurologic complications in adults. ZIKV is transmitted by mosquitoes of the Aedes species, which is the same vector for several other viruses, including Yellow fever virus, Chikungunya virus and Dengue virus. Currently, no vaccine or drugs are available to prevent or treat ZIKV infections. Our research was concentrated on finding the novel polymeric – curcumin conjugate to inhibit of ZIKV replication. We already demonstrated that poly(sodium 4-styrenesulfonate) (PSSNa) inhibits ZIKV replication in vitro both in animal and human cells, while no cytotoxicity is observed.[1] Curcumin (Cur) is the active component of dried root of Curcuma longa, an herb belonging to ginger family. Widespread research over last few decades has showed that curcumin is a potent anti-inflammatory agent with a powerful therapeutic potential against a variety of pathogens. To overcome low stability and poor bioavailability of curcumin, we synthesized Cur-PSSNa conjugates to both target and effectively inhibit ZIKV replication.

Experimental/Methods

Cur-PSSNa conjugates were prepared via reversible addition – fragmentation chain-transfer (RAFT) polymerization of 4-vinylbenzenesulfonate (SS) (Fig.1). The polymerization was carried out in mixture of dioxane and water at 90 °C with 4,4′-azobis(4-cyanovaleric acid) (ACVA) as thermal initiator. These conditions let to controlled reactions and generation of well-defined sulfonated oligomers/polymers with one curcumin molecule per polymer chain. The conversion of SS monomer after 4 h of polymerization was estimated from ^{1}H NMR measurements and found to reach about 50%. Cur-PSSNa conjugates were characterized by size exclusion chromatography calibrated with poly(sodium 4-styrenesulfonate) standards , using 0.1 M NaCl aqueous solution containing 20 v % acetonitrile as eluent (Table 1).

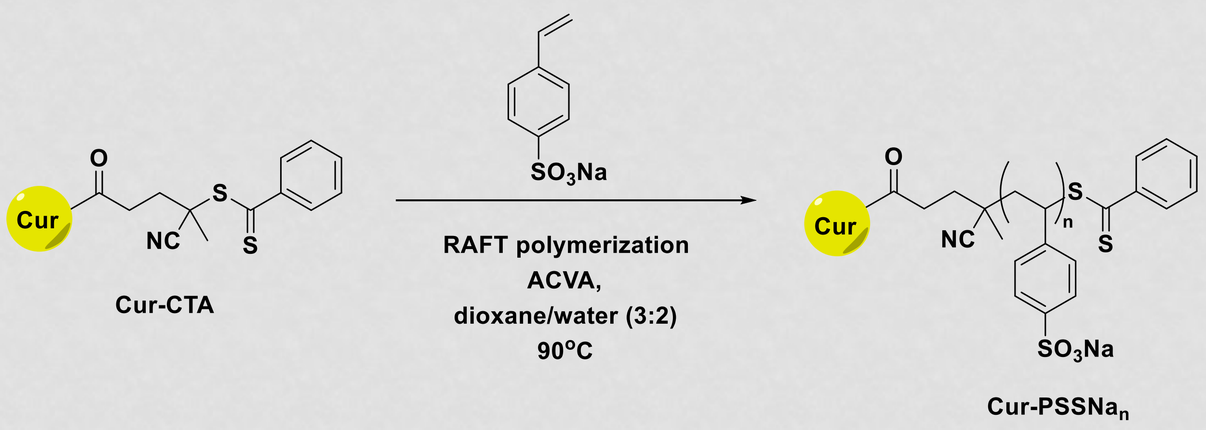

Figure 1. Synthesis of Cur-PSSNa conjugate

Table 1. Molecular characteristic of Cur-PSSNa polymers.

samples	M_n (theor)	M_n (GPC)	M_w (GPC)	PDI (GPC)	DP
Cur-PSSNa$_8$	2180		-	-	8
Cur-PSSNa$_{12}$	3720	3170	3620	1.14	12
Cur-PSSNa$_{26}$	6820	5900	7140	1.21	26
Cur-PSSNa$_{40}$	10940	8930	11300	1.26	40

Results

XTT assay was carried out to assess the cytotoxicity of Cur-PSSNa conjugate. Confluent Vero cells were incubated with solutions of obtained materials at various concentration for 3 days(Fig. 2).
We also performed virus inhibition assay to compare inhibitory effect of Cur-PSSNa with PSSNa with similar average molecular weight and curcumin itself (Fig. 3).

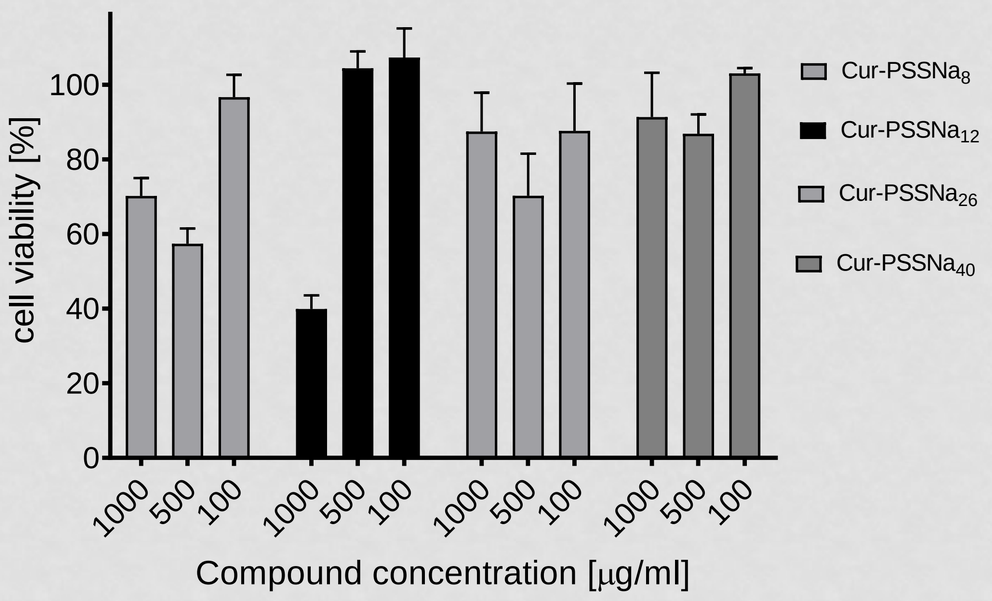

Figure 2. Cytotoxicity of Cur-PSSNa conjugate of average various molecular weight at 1000, 500 and 100 μg/ml. Results of XTT assay of tested polymers on Vero cells.

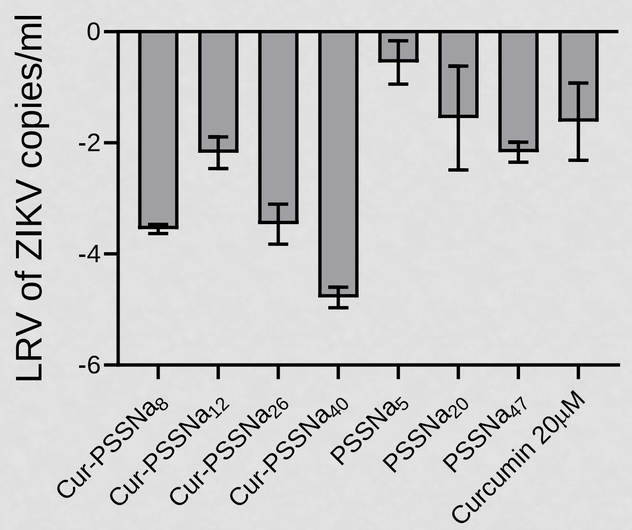

Figure 3. ZIKV inhibition assay carried out for Cur-PSSNa, PSSNa and Cur in Vero cells, infected with the virus in the presence of different polymers at the concentration of 100 μg/ml. Inhibition of the infection was evaluated using RT qPCR. Data are shown as the logarithmic decrease in the number of ZIKV copies in the samples compared to the control (copies of viral genome per milliliter).

Conclusion

Several curcumin – poly(sodium 4-styrenesulfonate) conjugates with different degree of SS polymerization (DP = 8, 12, 26 and 40) and narrow polydispersity index (PDI = 1.1 -1.3) was obtained. The tested polymers shown no toxicity at concentration 100 μg/ml.
Our preliminary results indicated that curcumin-PSSNa conjugate possess much stronger antiviral properties then PSSNa polymer of similar average molecular weight and curcumin itself.

Acknowledgements
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References
[1] P. Botwina, M. Obłoza, A. Szczepański, K. Szczubiałka, M. Nowakowska, K. Pyrć; **Viruses** 2020, 12, 926.