Anticancer activity of extended release loperamide from styrene-isoprene-styrene polymer *in vitro*

Jessica Winakor[1], Susan Wang[2], Rong Wang[3], George Sukenick[3], Ron Feiner[2], Declan Gwynne[2], Gregory W. Fischer[1], Michael G. Kharas[2,4,5], Daniel A. Heller[2,5], and Takeshi Irie[1,2,6#]

[1] Department of Anesthesiology & Critical Care Medicine, [2] Molecular Pharmacology Program, [3] Nuclear Magnetic Resonance (Analytical) Core Facility, [4] Center for Cell Engineering, Center for Stem Cell Biology, Center for Experimental Therapeutics, Memorial Sloan Kettering Cancer Center, New York, New York 10065 USA
[5] Weill Cornell Graduate School of Medical Sciences, [6] Department of Anesthesiology, Weill Cornell Medicine, New York, NY, 10065 United States

 # iriet@mskcc.org/irie.md.phd@gmail.com
X: @oncoanesthesia
Bluesky: @oncoanesthesia.bsky.social

Introduction

Sustained release drug delivery is a well established strategy to achieve durable pharmacologic effects. We recently developed styrene-isoprene-styrene (SIS) polymer as a drug release platform that largely retains the mechanical flexibility of the parent polymer, while allowing extended release of a number of drugs.[1] Here we studied release of loperamide, an over the counter anti-peristaltic treatment for diarrhea that works via peripheral mu opioid receptors. The drug also has anticancer[2, 3] as well as analgesic activity[4] and sustained release of loperamide may have clinical utility.

Methods

Loperamide HCl dose responses were in 1% DMSO. Cell titer glo 2.0 cell viability assays (Promega) were performed with murine *Kras*^{G12C}; *p53*^{null} double mutant non-small cell lung cancer (KP NSCLC) cell lines which recapitulates a human NSCLC, and murine MB49 urothelial cancers.

SIS was dissolved with or without loperamide HCl in chloroform, speed mixed (FlackTek), dried, and punched into 8mm diameter pellets.[1] Drug loaded versus blank pellets were eluted to aqueous solutions, eluates diluted with 2X media, then applied to live cells. Release of intact drug was confirmed by mass spectrometry of methanol eluates. Opioid receptor activity of released drug was confirmed with mu opioid receptor TANGO assays (a live cell bimolecular complementation assay)[5]. Statistical analyses used Prism 10.

Figure 1.

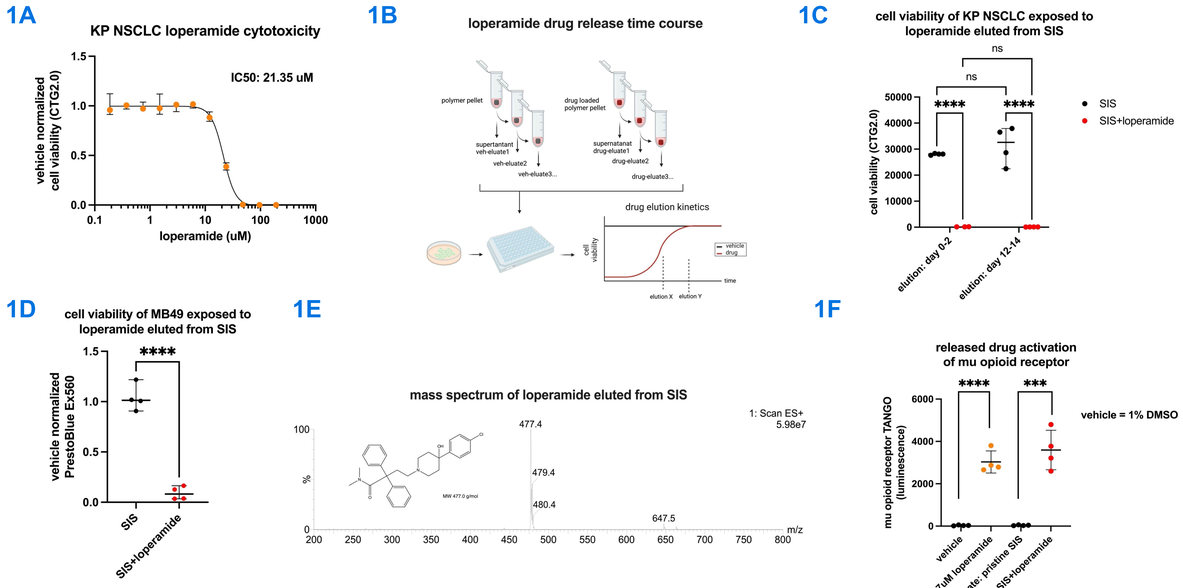

Figure 1 legend

1A. *Kras*^{G12C}*p53*^{null} non-small cell lung cancer (KP NSCLC) are loperamide sensitive
1B. Experimental workflow for *in vitro* cytotoxicity time course for Fig 1C.
1C. SIS-loperamide pellet eluates are cytotoxic against are against KP NSCLC cells durably over 2 weeks
1D. MB49 urothelial cells are also susceptible to SIS-loperamide eluate
1E. Mass spec confirmation of loperamide elution from SIS
1F. Live cell based mu opioid receptor TANGO assay confirms eluted loperamide retains activity against mu opioid receptor

Results

KP NSCLC cells treated 48h with loperamide HCl in 1% DMSO vehicle were assayed by cell titer glow revealing cytotoxicity IC50 of 21.4uM (Fig 1A). SIS-loperamide eluates were durably cytotoxic against KP NSCLC cells beyond 2 weeks (Fig 1B, 1C, two-way ANOVA overall p<0.0001). The loperamide cytotoxicity IC50 against MB49 urothelial cells was 18.7uM, and MB49 were also sensitive to SIS-loperamide eluate (Fig 1D). Release of intact loperamide was confirmed by mass spectrometry (Fig 1E), and released drug was confirmed active in the mu opioid receptor TANGO assay (Fig 1F).

Conclusion/Implications

SIS-loperamide HCl releases intact drug and eluates kill multiple cancer models *in vitro*, including KP NSCLC cells for more than 2 weeks, as well as MB49 urothelial cancer cells. Longer duration studies are needed to define the durability of loperamide release, and therapeutic indices evaluated. Extended release of loperamide from polymeric devices may be clinically useful in treatment of cancer.

Conflict of interest declaration:

none

References:

1. Feiner, et al. *ACS Appl Mater Interfaces*. 2022
2. Yoshida, et al. *Int J Mol Med*. 2000
3. Zhao, et al. *Cell Reports* 2022
4. Chung, et al. *Eur J Pain* 2012
5. Kroeze, et al. *Nat Struct Mol Biol*. 2015

Acknowledgements:

DH: NCI R01-CA215719, NINDS R01-NS116353, NSF CAREER Award 1752506, ACS Research Scholar GC230452; MK: NIDDK NIH R01-DK101989-01A1, NCI 1R01CA193842-01, R01HL135564, R01 CA274249-01A1, R01CA186702, R01CA283578, and R01CA225231-01; NCI Cancer Center grant P30 CA008748 to SV.