Reduced cardiotoxicity of spiro-tetrahydropyran bupivacaine analogues

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OVERVIEW

The goal of this study was to create active structural analogs of bupivacaine with reduced general and cardiotoxicity due to *in silico* modifications designed to affect their interaction with $Na_v1.5$ channel, which is believed to be the main target of bupivacaine toxicity.

INTRODUCTION

- Acute side effects of local anesthetics involve the cardiotoxicity believed to result from non-specific Na^+ channels blockade.
- There are nine distinct types of Na+ channels in humans .
- Toxicity of the currently marketed amide-type local anesthetics may be largely determined by their off-target pharmacology on various subtypes of sodium channels .
- Cardiotoxicity is caused by binding to $Na_v1.5$ channel, which is heart sodium channel, responsible for heart impulse distribution.

METHODS

Experimental animals. Adult male Balb/c mice aged 9-10 weeks were used in the study. Cardiotoxicity studies in isolated hearts were conducted on 18 adult male guinea pigs. All procedures were performed in accordance with guidelines for the use of experimental animals established by the institutional Committee on Animal Care and Use.

Test compounds. Bupivacaine and its spiro-cyclobutane (KL-1521), -tetrahydropyranoic (KL-1527) and -cyclopentane (KL-1536) analogues were synthesized by Custom Chemistry Department of Enamine Ltd.. Compounds had purity of >95% by HPLC-UV 215 nm, 254nm and ELSD, confirmed by either LC/MS and 1H-NMR.

Tail flick assay. Thermal sensitivity was determined by using a modification of the method described by D'Amour and Smith (1941). The level of analgesia for each mouse was measured by the tail-flick assay using analgesia meter (Columbus Instruments, USA).

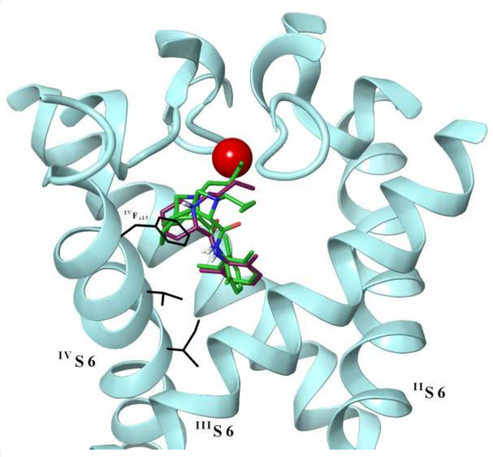
LD50. To determine the median lethal dose, mice were intravenously treated with the test substances at several graduated doses (6-8 groups of at least 5 mice for every compound). Before treatment, mice were fasted for 3-4 hours with *ad libitum* access to water. Sublethal dose had been defined as the highest concentration, which gave 100% survival during the LD50 studies. LD50 was determined by probit analysis using BioStat v5 software.

Toxicity study. Toxic effects of all test compounds after subcutaneous administration and at sublethal IV doses (5 mg/kg for Bupivacaine, 5.5 mg/kg for KL1521, 31 mg/kg for KL1527 and 12 mg/kg for KL1536) were evaluated during 14 days after a single treatment by daily recording of the body weight and visual toxic signs according to the standard procedures. Relative organs weights (kidneys, adrenal glands, thymus, spleen, lungs, testicles, heart, pancreas and liver) were determined as the ratio of organ weight to the whole body weight and expressed in percents.

Langendorff preparation of isolated heart perfused at constant flow. The animals were anesthetized, the animal thorax was opened and the heart removed and quickly mounted on a non-recirculating Langendorff apparatus, and coronary arteries were perfused *via* the aorta. Electrocardiogram (ECG), the pressure in the aorta and in the left ventricle was measured.

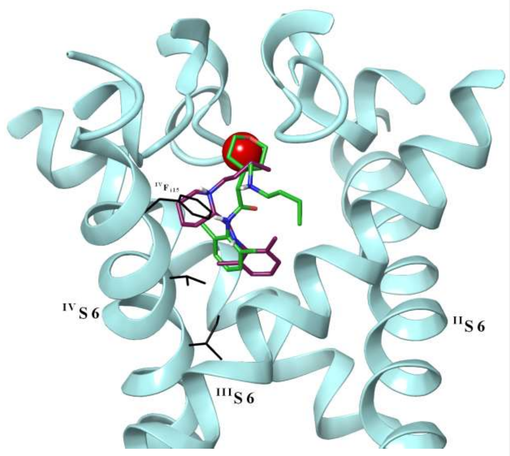
RESULTS

There is no significant difference in binding poses between bupivacaine, KL-1531 and KL-1536. However, compound KL-1527 has different interaction mode, which is observed during docking studies with the open form of Nav1.5 channel in presence of Na ions.

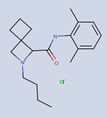
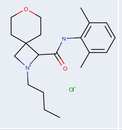
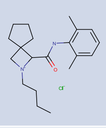
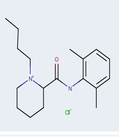


Docking of **bupivacaine** and compounds **KL-1521**, **KL-1536** in $Na_v1.5$ channel

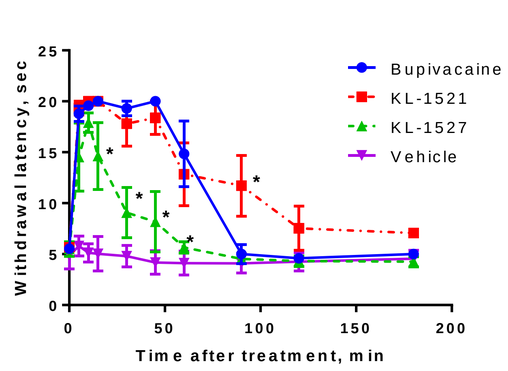
VS.

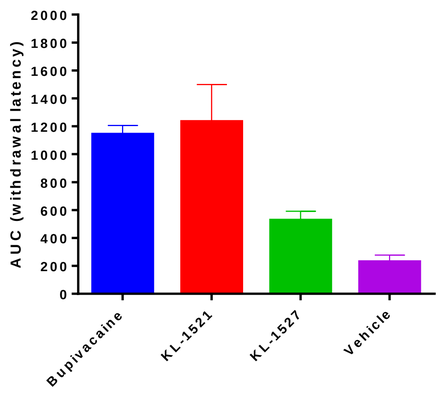


Docking of **bupivacaine** and compound **KL-1527** in $Na_v1.5$ channel

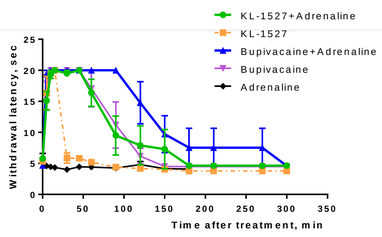
Compound ID	Structure	logD, PBS pH7.5	Plasma Protein Binding (mouse),%	Microsomal stability (mouse), T1/2, min	Solubility, PBS pH 7.5, uM	pKa calc. (ChemAxon)	hERG Predictor™ binding at 25 µM,%
%KL-1521		4	94.9	8	340	7.21; 13.60	33
KL-1527		3.3	89.9	20	>400	7.38; 13.60	12
KL-1536		4.23	98.2	6	220	7.45; 13.60	38
Bupivacaine		2.69	89.3	26	>400, (~600 µM at pH 8-12, Shah 1992)	8.0; 13.62	20

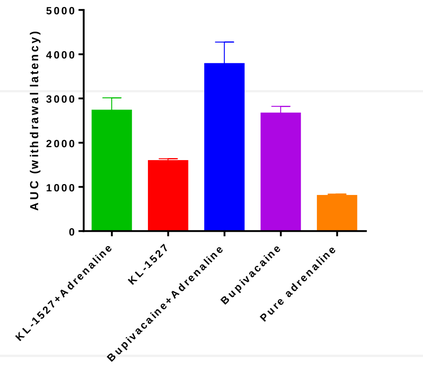
Antinociceptive effect of bupivacaine analogues





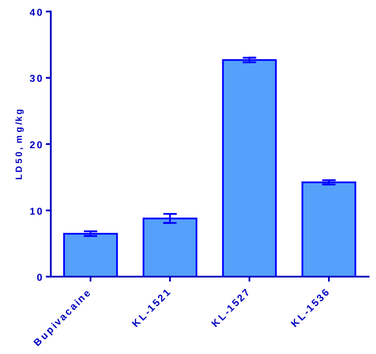
Time course of antinociception, produced by bupivacaine analogues in tail-flick assay, administered at a dose of 0.5 mg/mice, and its AUC evaluation.



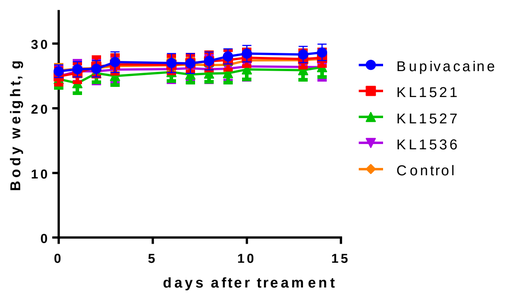


Tail withdrawal latency of mice, treated with 0.5 mg/mice KL-1527 or bupivacaine in combination with adrenaline (0.5ug/mice) and its AUC evaluation.

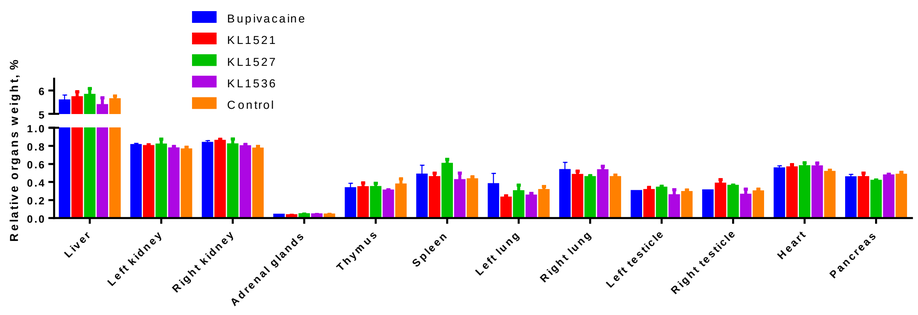
Toxicity studies



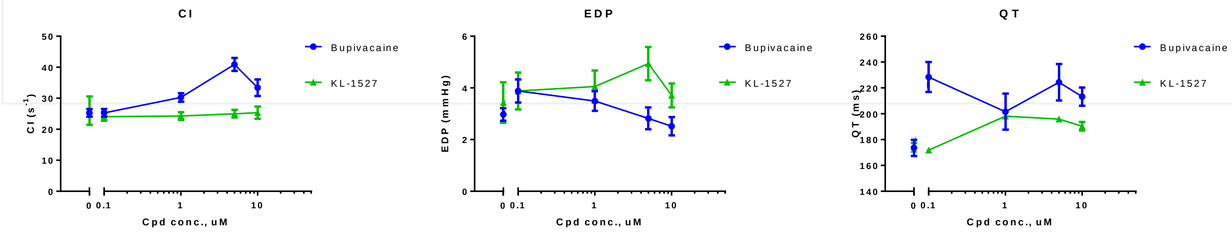
General toxicity. Median lethal dose of bupivacaine analogues (intravenous route of administration).



Dynamics of mice body weight gain during 14 days after bupivacaine analogues administration in sublethal doses.



Relative organs weights on the 14-th day after bupivacaine analogues administration in sublethal doses.



Comparing the cardiotoxicity (chronotropic and inotropic effects) of bupivacaine and its analogue in the Langendorff isolated heart model.
CI contractility index, s^{-1}
EDP end-diastolic pressure, mm Hg
QT interval from of the Q wave peak interval to the end of the T peak, ms

CONCLUSIONS

- ✓ Novel structural analogs of bupivacaine have comparable antinociceptive action, but substantially reduced general and cardiac toxicity.
- ✓ Activation of $Na_v1.5$ channels, expressed in the heart tissue, leads to a rapid cell membrane depolarization. This is a key event, which initializes action potential, hence any compounds inhibiting this channel may demonstrate both inotropic as well as chronotropic effects on heart. Compound KL-1527 effects on isolated heart are significantly lower comparing to the bupivacaine.