Controlling the rate of entry to the CNS by polymer conjugation

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Abstract

The blood-brain barrier (BBB) limits access of hydrophilic and large molecules to the CNS, but remains permeable to many small hydrophobic molecules allowing psychoactive drugs to reach their target. However, for some drugs, rapid entry across the BBB leads to high peak concentrations that trigger undesirable effects. In other cases, drugs with primary sites of action in the periphery also have CNS binding sites which cause central side effects, adversely affecting their clinical utility. Here, we present an approach to controlling the entry of drugs to the CNS by using directed polymer conjugation. Since this strategy is compatible with, and often enhances, oral absorption and can be tuned to enable different rates and degrees of transfer across the BBB, it provides several advantages over other CNS-exclusion technologies which rely on incorporation of a charged group or a peptide linkage to limit membrane permeability.

Clinical efficacy for the use of a polymer in restricting access to the CNS was previously demonstrated with NKTR-118, oral PEG-naloxol, which acts at peripheral opioid receptors to relieve opioid-induced constipation without reversing centrally mediated analgesia. To demonstrate the broad applicability of this approach, we have now utilized directed polymer conjugation on a group of structurally and pharmacologically diverse molecules and have investigated the effect on CNS penetration.

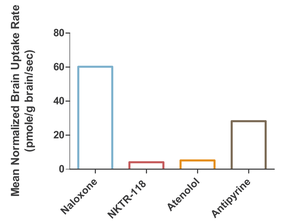
Molecules containing polymer side chains were administered intravenously to rats and the ratios of their concentrations in brain versus plasma were determined after 1 hour. For all the polymer conjugates, concentrations in plasma were higher than in the brain, unlike the control compounds which all produced higher levels in the brain. Doxepin, (a tricyclic antidepressant), diphenhydramine (an ethanolamine antihistamine), promethazine (a phenothiazine antihistamine) and nalbuphine (an opioid agonist-antagonist) displayed brain:plasma ratios of 28:1, 19:1, 40:1 and 4:1 respectively. By contrast, polymer conjugates from these chemical classes produced corresponding ratios of 1:6, 1:5, 1:9 and 1:13 demonstrating a dramatic reduction in CNS penetration.

To enable slowed uptake to the brain, a range of opioid compounds containing different polymer conjugates was generated. Brain uptake rates varied from to 29 to 0.7 uL/g/min in an *in situ* brain perfusion model in rats, and were slower than the uptake rate of 148 uL/g/min exhibited by a control opioid. This polymer conjugation technology therefore provides a versatile tool for optimizing the permeability across the BBB to control the rate of CNS entry for diverse molecules from different pharmacological classes.

Results

Polymer conjugation restricts NKTR-118 to the periphery

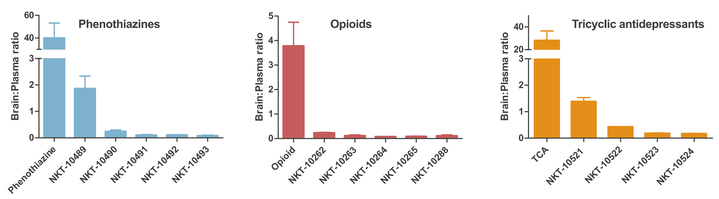
NKTR-118 displays a significantly reduced brain uptake rate



The rates of brain uptake of naloxone and NKTR-118 were measured using the *in situ* brain perfusion model in rats. Naloxone rapidly entered the brain at a rate comparable to the fast permeation reference drug, antipyrine. The rate of brain uptake of NKTR-118 was significantly slower and was comparable to that of the low permeation reference drug, atenolol.

Structurally diverse molecules can be restricted from the CNS using polymer conjugation

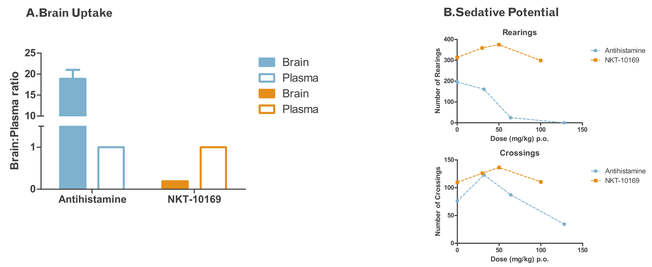
Brain levels of multiple classes of compounds are reduced following polymer conjugation



Sprague-Dawley rats were dosed intravenously with various compounds at concentrations of 5-10 mg/kg . Brains and plasma were collected one hour post-dose. Concentrations of test articles in brain and plasma were measured using LC-MS/MS. The ratio of the concentration of each test article brain (ng/g) versus plasma (ng/mL) is expressed as the brain: plasma ratio. Each data point is Mean +/- SD of 3 rats.

Reduced brain entry improves the side-effect profile of an antihistamine

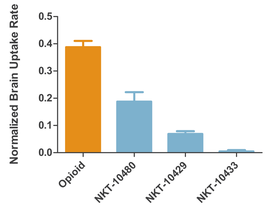
Reduced brain entry provides an antihistamine with reduced sedative/stimulant side effects



A. The brain:plasma ratios of a polymer antihistamine conjugate and a marketed first generation antihistamine were measured 1 hour after intravenous dosing to rats. Bars represent Mean ± SD of 3 rats.
B. The sedative/stimulant activities a polymer antihistamine conjugate and a first generation antihistamine were evaluated in an automated activity meter test in mice. Mice (n=10 per dose group) were dosed orally with the indicated concentrations of test compounds one hour prior to observation. Cumulative crossings (lower panel) and rearings (upper panel) were automatically recorded over 40 minutes of observation. Data are represented as the percentage change in activity over vehicle-treated animals.

Brain uptake rate can be tuned through choice of polymer conjugation

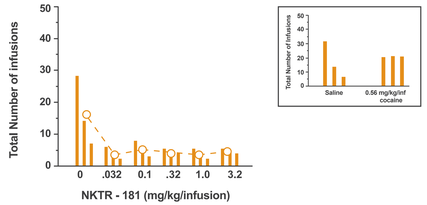
Polymer conjugated opioids with varying brain uptake rates can be generated from a single scaffold



The brain uptake rates of a series of opioids with differing polymer side chains were measured using the *in situ* brain perfusion model in rats. Following a 30-sec brain perfusion with 10μM of test compounds via the left carotid artery of rats, the concentration of test articles in the brain were measured using LC-MS/MS. The unidirectional brain permeability constant, Kin (mL/g/min) was calculated for each compound and normalized to that obtained with the high permeability control, antipyrine. Bars are Mean ± SD of 3 rats.

Slowed brain uptake provides an opioid with low abuse liability

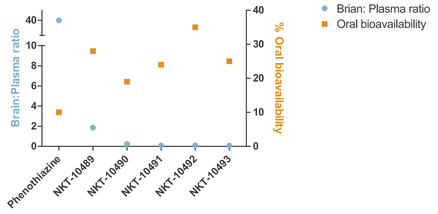
NKTR-181 shows weak reinforcing activity in a 3-day substitution test in rats



Self-administration studies were performed on cocaine-trained rats, involving intravenous bolus infusions of NKTR-181, cocaine (COC) or saline (SAL) at the indicated unit doses. In the 3-day substitution test a lever-press protocol (FRI0) was used in 1 hour sessions on 3 consecutive days, with reinforcement defined as <20% variability over 3 sessions. NKTR-181 shows no reinforcing activity up to the highest dose tested. Saline and cocaine (inset) display the expected behavior for negative and positive reinforcement respectively.

Peripherally restricted drug conjugates possess high oral bioavailability

Polymer phenothiazine conjugates with restricted CNS entry retain high oral bioavailability



A series of polymer conjugates generated from an identical scaffold were administered to rats at oral doses of 5 mg/kg. Plasma was sampled at various time points following dosing and concentrations of test compounds in plasma were measured using LC-MS/MS. Oral bioavailability values were calculated from dose-normalized AUC values obtained following oral and intravenous dosing of test compounds. Brain: Plasma ratios were measured in rats 1 hour following an IV dose.

Methods

Brain uptake rate (*in situ* perfusion in rats)

The rate of brain uptake of test compounds was measured using an *in situ* brain perfusion method in Sprague Dawley rats. The left common carotid artery was cannulated and test compounds in Kreb's Ringer buffer were perfused for 30 seconds. The left brain hemisphere was excised, homogenized and the concentrations of test articles were measured using LC-MS/MS. The unidirectional brain permeability, Kin, was calculated as Kin = [Cbr/Cpf]/t, Where Cbr and Cpf represent the concentration of compound in brain and perfusate respectively and t is the perfusion time.

Brain to plasma ratios in rats

Test compounds were administered intravenously to rats via the tail vein. One hour following dosing, the animals were euthanized, exsanguinated and concentrations of test articles in brain and plasma samples were measured using LC-MS/MS. Brain: Plasma ratios are calculated from the concentrations in brain to that measured in plasma.

Activity meter test in mice

Mice were placed in an activity meter which consists of a covered plexiglass cage equipped with photocell assemblies at defined positions above the floor of the cage to automatically record activity and rearing activity. Scores for activity and rearing were recorded by a computer in 10 minute time frames and cumulated over 40 minutes. Test compounds were administered as a single oral dose, one hour prior to observation.

Conclusions

- Polymer conjugation provides a novel approach to controlling the rate of brain entry of drugs
  - To reduce dose-limiting central side effects
  - Enabling novel therapies for drugs with peripheral targets, but unacceptable central toxicities
- PEG conjugates retain good oral bioavailability, despite significant CNS exclusion
- The rate of brain uptake can be tuned through choice of PEG chemistry
- PEG conjugation represents a versatile technology that can be successfully applied to control the rate of brain entry of molecules from diverse chemical classes
- The polymer conjugation technology has been clinically validated with NKTR-118, NKTR-119 and is being applied to a research pipeline of mechanistically diverse analgesics for acute and neuropathic pain

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To download a copy of this poster, please visit http://www.nektar.com

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