Kv1.3 Ion Channel Blockers as Novel, Oral Therapies for Multiple Sclerosis

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Background

Kv1.3: A drug target for the treatment of multiple sclerosis and other autoimmune diseases

Multiple Sclerosis (MS) is an autoimmune disease characterized by axonal demyelination in the central nervous system (CNS) which results in a myriad of debilitating neurological symptoms. The initial stages of MS are associated with episodes of CNS inflammation followed by periods of remission where recovery due to remyelination is either partial or complete. Eventually, the disease enters a secondary progressive stage associated with axon loss. The majority of the MS drug market is dominated by the ABC treatments (Avonex, Betaseron and Copaxone) which slow the onset of clinically defined MS and reduce the severity and rate of relapses. All treatments are currently administered either by injection or IV infusion. A tremendous opportunity exists therefore for an orally administered, drug with fewer side effects

The Kv1.3 (KCNA3) ion channel is a novel drug target shown to be crucial for the activation and proliferation of autoantigen - specific Effector Memory T (TEM) cells which have been implicated in the pathogenesis of numerous autoimmune diseases (1). Proof of concept studies in animal models have demonstrated the strong potential of Kv1.3 blockers as treatments for Multiple Sclerosis, Rheumatoid Arthritis, Type-1 Diabetes (2) and Psoriasis (3).

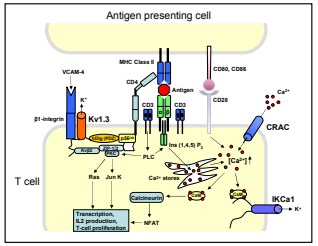


Figure 1. *The role of Kv1.3 in T cell proliferation.* Upon antigenic stimulation, both Kv1.3 and IKCa1 (intermediate conductance Ca^{2+}-activated K^+ channel) maintain membrane potential which allows a constant Ca^{2+} influx through calcium release activated channels (CRAC). In chronically activated memory T cells, the expression of Kv1.3 increases and IKCa1 decreases, making these cells selectively sensitive to Kv1.3 blockade (modified from Chandy et al 2004, ref # 4).

Bionomics Kv1.3 program

Bionomics has undertaken a medicinal chemistry effort in order to identify small molecule blockers of Kv1.3 for further development as therapeutics for Multiple Sclerosis and other autoimmune disease. Our medicinal chemistry effort has been based primarily on *Khellinone*, a plant natural product which blocks Kv1.3 currents with an EC_{50} of ~10 µM.

This effort has yielded a number of compounds that exhibit significantly improved potency and efficacy as well as, improved physicochemical properties that make them amenable to oral administration. Currently, BNC245 is the most advanced lead in Bionomics Kv1.3 blocker compound series.

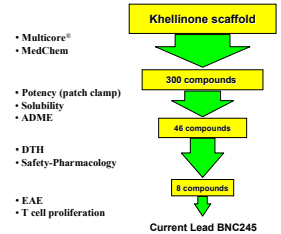
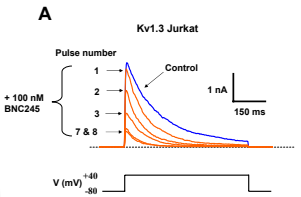


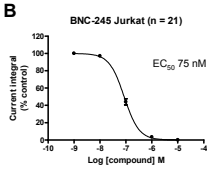
Figure 2. *Overview of the Bionomics Kv1.3 blocker drug discovery program.* Khellinone analogs showing suitable Kv1.3 potency and drug like properties were initially tested for efficacy in the rat Delayed Type Hypersensisitivity (DTH) model. Selected compounds were then tested for efficacy in the more MS disease-specific rat experimental autoimmune encephalomyelitis (EAE) model.

Results

1. BNC245 blocks Kv1.3 currents in Jurkat cells

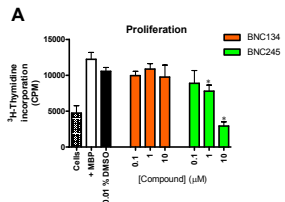


A. Use-dependent block of whole-cell Kv1.3 currents recorded in a Jurkat cell by 100 nM BNC245. Currents were activated by 500 ms voltage steps to +40 mV from a holding voltage of -80 mV every 30 s.

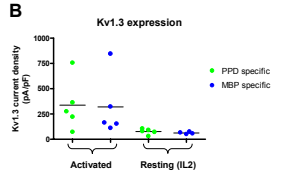


B. BNC245 dose -response obtained in Jurkat cells

2. BNC245 inhibits proliferation of rat encephalitogenic T cells which express high levels of Kv1.3

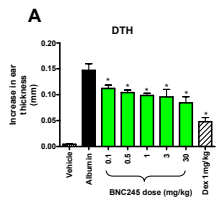


A. BNC245 potently inhibited the proliferation of freshly activated MBP-specific TEM cells in a dose-dependent manner. These cells are encephalitogenic in rats. BNC134, a structurally similar analog with no Kv1.3 blocking activity did not affect proliferation. Results show mean ± 1 SD, n = 3 wells, * $p < 0.05$.

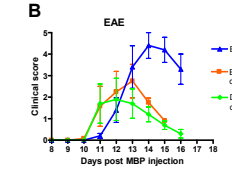


B. Freshly activated MBP-specific T cells have increased Kv1.3 expression. The high Kv1.3 phenotype is typical of activated TEM cells but not antigen specific. Filled circles represent data from individual cells.

3. BNC245 reduces DTH and treats EAE in Lewis rats



A. BNC245 administered as a single oral dose effectively reduces the DTH response in Lewis rats. Dexamethasone was given SC for 2 days. Results show mean ± 1 SEM, n = 5 rats, * $P < 0.05$.



B. BNC245 administered as a single oral daily dose of 30mg/kg for 8 days effectively reduces the severity of EAE, an animal model of MS. Results show mean clinical score ± 1 SEM (n = 7 rats/ treatment group).

Neurological scoring : 0 = no abnormality; 1 = distal weakness of the tail; 2 = complete weakness of the tail; 3 = mild weakness in one or two hindlimbs; 4 = moderate paraparesia of one or two hindlimbs; 5 = total paraplegia.

4. BNC245 summary of PK properties

Route of admin	ip	po
Dose (mg/kg)	34	30
$t_{1/2}$ (h)	5	5
Cmax (µM)	8.7	9.2
Tmax (min)	15	60
AUC (mM*min*kg)	28	36
BA%	71	90

5. BNC245 is well tolerated

Acute dosing in rat
- No observable clinical signs, organ toxicities or haematology at 500 mg/kg

7-day repeat dosing in rats
- No observable clinical signs, organ toxicities or haematology at 100 mg/kg

ECG assessment in Guinea pigs
- No drug related changes observed following cumulative dosing to 60mg/kg

6. Recent analogs

Parameter	BNC245	BNC440	BNC441
Kv1.3 EC_{50} (Jurkat) nM	75	3	41.9
*E_H (Hum)	0.94	0.79	0.71
DTH (PO)	+++	+++	+++
Selectivity (fold) Kv1.3/Kv1.1	3.3-4.6	7.5-16.3	8.5-18
Selectivity (fold) Kv1.3/Kv1.5	3.17-4.6	77-137	24-39

*E_H = Human liver microsome predicted hepatic extraction ratio

Methods

Electrophysiology

Kv1.3 currents were measured in Jurkat cells and activated and propagated rat MBP and PPD-specific T cells using a planar chip version of the whole-cell patch clamp technique (Nanion Technologies, GmbH). Cells were bathed in a solution containing (mM): 160 NaCl, 4.5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 5 Glucose, 10 HEPES pH 7.4 (NaOH), 340 mOsm.Kg^{-1}. Internal solution contained (mM): 75 KCl, 10 NaCl, 70 KF, 2 $MgCl_2$, 10 EGTA, 10 HEPES pH 7.2 (KOH), 320 mOsm.Kg^{-1}. Whole-cell Kv1.3 current transients were activated by 500 ms depolarizing voltage pulses to + 40 mV from a holding potential of -80 mV. Activating voltage pulses were applied at 30 s intervals to allow complete recovery from slow C type inactivation. For Kv1.3 block EC50 determination, compounds were diluted in bath solution (0.5% DMSO final) and pipetted into to the recording bath at increasing concentrations. Kv1.3 currents were allowed to reach steady state in between compound additions. The final current integral obtained at each compound concentration was normalized to the control and EC_{50} values were generated by fitting variable-slope sigmoidal dose response curves to the data using Graphpad Prism software.

Proliferation studies

MBP-specific T cell lines were activated with MBP in the presence of APC and BNC134 or BNC245 (100 nM, 1 µM or 10 µM) or 0.01% DMSO (solvent control) for 3 days. ^{3}H-Thymidine was added for the last 15 h and the proliferation measured as incorporation of ^{3}H-Thymidine

Delayed Type Hypersensitivity (DTH)

Lewis rats (8 weeks old) were immunized at the base of the tail with 200 µl of an emulsion of egg albumin grade II (Sigma) in complete Freund's adjuvant (Sigma). The emulsion was prepared with 50 % CFA and 50% saline containing 1 mg/ml albumin. Control group received equivalent amount of emulsion in which albumin was omitted. Seven days later, rats were challenged by an injection of 10 µl of albumin (2 mg/ml) dissolved in saline in the right pinna ear. Ear swelling was measured 24 hr after albumin challenge in anaesthetized rats (ketamine 60 mg/kg + xylazine 4 mg/kg). Values are given as differences in the thickness (mm) of the albumin injected ear measured before the challenge and after the challenge.

Experimental Autoimmune Encephalomyelitis (EAE)

Nine week-old female Lewis rats (Janvier, Le Genest-St-Isle, France) were used. Rats were anaesthetized by 60 mg/kg of ketamine (Imalgene500®, Rhône Mérieux, Lyon, France) + 4 mg/kg of xylazine (Rompum 2%, Bayer Pharma, Kiel, Germany). Rats were then injected in the hind footpad with a total volume of 100 µl of inoculum per paw, consisting of 100 µg of myelin basic protein (MBP) (Sigma, L'Isle d'Abeau Chesnes, France) emulsified (1:1) with Incomplete Freund's adjuvant (IFA) (Sigma, L'Isle d'Abeau Chesnes, France) containing 500 µg of heat-inactivated mycobacterium tuberculosis (strain H 37 RA from Difco). Control rats received equivalent volume of inoculum free of MBP.

Conclusions

1. **BNC245 is a novel potent blocker of the Kv1.3 ion channel that inhibits the proliferation of encephalitogenic T cells and displays good oral efficacy in animal inflammatory disease models with no side effects**
2. **The effectiveness of BNC245 in EAE demonstrate the potential of Bionomics Kv1.3 blockers for further development as an oral MS treatment**
3. **An ongoing medchem program has recently produced more metabolically stable analogs that display greater potency for Kv1.3 and selectivity over other kv1.x channels.**

References

1. Beeton et al (2005) Mol Pharmacol 67 : 1369-1381.
2. Beeton et al (2006) PNAS 103: 17414-17419.
3. Azam et al (2007) J Inv Derm : 1-11.
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