TRPA1 as a drug Target
Overview

- TRPA1 – an introduction
- Validation as a target for drug discovery
  - For migraine
  - For respiratory disorders
  - For inflammatory and neuropathic pain
- Medicinal chemistry of TRPA1 antagonists
TRP channels play diverse (patho)physiological roles

**Respiratory**
- TRPC6, TRPV1, V4, TRPA1, TRPM8

**Kidney**
- TRPC6, TRPM6, TRPP2

**Gastrointestinal**
- TRPV1, V2, V4, TRPM8, TRPA1

**Neuronal**
- TRPC3, C5, C6
- TRPM2, M4, M7, ML1

**Skin**
- TRPV1, V3, TRPA1

**Pain**
- TRPV1, V3, V4, TRPM3, M8, TRPA1

*Also bladder disorders, diabetes*
TRPA1 introduction

- Member of the transient receptor potential (TRP) superfamily of ion channels
- Characterised by extended chain of repeating ankyrin repeat domains
- Non-selective cation channel; carries both monovalent and divalent cations
  - TRPA1 activation can drive both depolarisation and calcium signalling
- Multiple modes of activation
  - Low temperatures – *still some controversy/species differences*?
  - Mechanical pressure – *still some controversy*?
  - Exogenous mediators such as airborne irritants
  - Endogenous mediators such as reactive oxygen species, inflammatory mediators
- Expressed in many different tissue beds and implicated in many disease areas
TRPA1: A chemosensitive ion channel

Chen, Arch Pharm, 2015

FEPS: familial episodic pain syndrome
TRPA1 receptors integrate signals from multiple sources
Nociception and neurogenic inflammation

- TRPA1 agonists activate channel
- GPCR dependent activation of PLC reduces TRPA1 ‘brake’ by PIP2
- PLC elevation triggers Ca\(^{2+}\) release from stores which may increase TRPA1 Ca\(^{2+}\) flux
- Ca\(^{2+}\) influx via TRPV1 also increases TRPA1 activity
- TRPA1 also activated by:
  - Various migraine – causing irritants
  - Methylglyoxal: implications for diabetic neuropathic pain
  - Formalin: mechanistic basis for a common inflammatory pain model
- Heightened intracellular Ca\(^{2+}\) levels trigger release of pro-inflammatory neuropeptides, such as substance P or CGRP, initiating neurogenic inflammation

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TRPA1: role in migraine
Activators of TRPA1 cause migraine

- Migraine is triggered by CGRP release from trigeminal neurons, which express TRPA1
- Exogenous or endogenous molecules that act as triggers for migraine activate TRPA1
- Californian ‘headache tree’ the bay laurel *Umbellularia californica* releases the reactive ketone umbellulone, which activates TRPA1

![Chemical structures]

- Umbellulone
- Thiol adduct
- Verbenone
- Piperitone
TRPA1: role in migraine
Activators of TRPA1 cause migraine

- No effect on TRPV1 or TRPM8
- Umbellulone activation of TRPA1 evokes currents in trigeminal ganglion neurones
- Umbellulone releases CGRP from rat spinal cord and evokes an increase in meningeal blood flow

Nassini, Brain, 2012
TRPA1: role in migraine

TRPA1 desensitisers protect against migraine

- Feverfew is used to treat pain, headaches and migraine
- The natural product parthenolide (isolated from feverfew) is a partial agonist at TRPA1
- Initial activation of TRPA1 develops into desensitisation and reduced neuropeptide release

Nassini, Pain, 2013
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Nassini, Pain, 2013
Role of endogenous and exogenous agents that modulate TRPA1 and play a role in migraine

Benemei, BJP, 2013
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TRPA1: role in respiratory disorders

- TRPA1 is expressed in lung fibroblasts, epithelial cells, and c-type neurons
- Activated by airborne irritants such as cigarette smoke, acrolein

- TRPA1 is implicated in cough following exposure to respiratory irritants, bronchoconstriction/airway hyper-responsiveness leading to asthma, and neurogenic inflammation leading to COPD
  - The receptor is activated by inflammatory mediators present in the lung such as bradykinin, histamine, prostanoids, as well as reactive oxygen species
  - TRPA1 plays a crucial role in airway inflammation and the late asthmatic response
  - TRPA1 integrates interactions between the immune and nervous systems in the airways

- TRPA1 antagonists could be effective in diseases such as asthma, COPD and rhinitis
TRPA1: role in respiratory disorders

TRPA1 is activated by many airway irritants

Isocyanates induce lacrimation, pain, airway irritation, and edema

Similar responses are elicited by chemicals used as tear gases

Other irritants such as acrolein, which is present in cigarette smoke, also activate TRPA1

CSE triggers trachea plasma extravasation

CS = CS tear gas
CN = CN tear gas
CR = CR tear gas
PS = PS tear gas
BenzBr = Benzyl Bromide
BrAc = Bromoacetone

Bessac. FASEB J 2009
Bandell. Neuron 2004
TRPA1: role in respiratory disorders
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Bessac. *FASEB J* 2009
Bandell. Neuron 2004
TRPA1: role in respiratory disorders

NAPQI increases neuropeptide release through TRPA1

- Epidemiological evidence has associated use of therapeutic Acetaminophen (APAP) doses with an increased risk of COPD and asthma
- The metabolite of APAP (NAPQI) activates TRPA1 and causes
  - the release of pro-inflammatory neuropeptides from sensory nerve terminals in rodent airways, thereby initiating neurogenic edema and neutrophilia

TRPA1: role in respiratory disorders

NAPQI increases airway inflammation through TRPA1

- Repeated therapeutic APAP doses to mice increased neutrophil numbers, myeloperoxidase activity, and cytokine and chemokine levels in the airways
  - ablated by TRPA1 inhibition (HC-030031) and knockout of TRPA1

TRPA1: role in respiratory disorders

TRPA1 is activated by reactive oxygen species

- During inflammation reactive oxygen species are generated by infiltrating macrophages and neutrophils
  - Hypochlorite is generated through myeloperoxidase mediated catalysis in neutrophils
  - During inflammation hypochlorite concentrations exceed those required for TRPA1 activation
- TRPA1 knockout mice show large reduction in airway responsiveness to hydrogen peroxide administered by aerosol
- Antigenic pollen proteins induce ROS production in allergic airway inflammation

Boldogh. J Clin Invest, 2005
TRPA1: role in respiratory disorders
KO of TRPA1 reduces symptoms in a mouse asthma model

Number of inflammatory cells in bronchoalveolar lavage fluid (BALF) from mice challenged with ovalbumin with/without TRPA1

- Genetic silencing of TRPA1 significantly reduced the number of inflammatory cells & cytokines in BALF from mice challenged with ovalbumin
- Genetic silencing of TRPA1 significantly reduced airway resistance produced by i.v. acetylcholine in mice challenged with ovalbumin

Caceres. PNAS (2009) PMID: 19458046
TRPA1: role in respiratory disorders
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**TRPA1: role in respiratory disorders**

TRP receptors are implicated in bradykinin and prostaglandin responses in the airways

- Bradykinin and PGE2 trigger depolarisation in GP vagus
- Signal is inhibited by TRPA1 antagonists such as HC-030031, as well as TRPV1 antagonists
- Depolarisation evoked by airway irritants is blocked by TRPA1 antagonists
- Further roles for TRPM8 (menthol) and TRPV4 (citric acid)
TRPA1: role in respiratory disorders

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TRPA1: role in pain signalling
Strong validation for the therapeutic potential of TRPA1 antagonists

- **Expression profile**
  - High expression in small/medium sized peptidergic fibres (co-expressed with TRPV1)
  - TRPA1 is upregulated in nerve injury and inflammatory pain models

- **Genetic rationale**
  - Gain of function TRPA1 mutation associated with familial episodic pain syndrome
  - TRPA KO has reduced response to formalin-induced pain, bradykinin induced mechanical hyperalgesia / nocifensive behaviours and suprathreshold mechanical stimulation
  - TRPA1 antisense reduced cold allodynia in a neuropathic (SNL) and inflammatory pain model (CFA)

- **Human and rodent pathophysiology linked to elevated TRPA1 agonists**
  - Exogenous mediators: formaldehyde, cinnamaldehyde, AITC, acrolein cause pain in humans and rodents
  - 4-HNE increased in osteoarthritis; methylglyoxal increased in diabetic peripheral neuropathy

- **Human and rodent pharmacological evidence**
  - TRPA1 antagonists reverse pain scores in diverse animal models
  - [Efficacy of Glenmark GRC17536 in diabetic neuropathy patients](#)
TRPA1: expression and function in pain signaling

1. Activated by inflammatory mediators and molecules released during tissue damage
2. Elicits generator potential
3. Localization at pre-synapse and effects on transmitter release
4. Flare – *basis for a straightforward clinical biomarker?*
5. Itch
TRPA1: a rare genetic channelopathy validates the target
Familial episodic pain syndrome (FEPS)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (17 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain severity (0-10)</td>
<td>9.7</td>
</tr>
<tr>
<td>Min duration</td>
<td>34 min.</td>
</tr>
<tr>
<td>Max duration</td>
<td>62 min.</td>
</tr>
<tr>
<td>Triggers</td>
<td>Fasting (100%), Cold (71%), Exercise (71%), Illness (47%)</td>
</tr>
<tr>
<td>Location of pain</td>
<td>Shoulders/Arms (47%), Thorax (35%), Abdomen (12%), Whole body (12%)</td>
</tr>
</tbody>
</table>

Rare gain of function mutation leading to increased TRPA1 current and a severe pain phenotype

Reactive pro-algesic mediators – hydroxynonenal
4-Hydroxy-2-nonenal (HNE) is produced when ROS peroxidate phospholipids
Reactive pro-algesic mediators – hydroxynonenal

Hydroxynonenal activates TRPA1

- HNE activates the tet-induced TRPA1 channel in HEK293 cells expressing rTRPA1
- Effect is blocked by ruthenium red
- HNE triggers release of neuropeptides substance P and CGRP from central and peripheral nerves and promotes extravasation
- HNE induces tactile allodynia in the rat hind paw
Reactive pro-algesic mediators – methylglyoxal

Methylglyoxal (MG) and diabetic peripheral neuropathy (DPN) in diabetes patients

Plasma levels of MG are correlated with pain in DPN

TRPA1 activation by MG in diabetes

Reactive pro-algesic mediators – methylglyoxal
Methylglyoxal induced acute and chronic pain is TRPA1 dependent

MG induces acute nocifensive behaviours

Elevated MG levels induced by GLO1 inhibition induce hyperalgesia

TRPA1 antagonists are active in multiple pain models
AP-00750 inhibits PF-4840154-induced mechanical alldynia

Compounds were administered 1 hour prior to the intraplantar injection PF-4840154 (0.05nmol/50µl/paw),

*, p<0.05 vs respective vehicle-treated group (Student’s t-test).
TRPA1 antagonists are active in multiple pain models
AP-00750 inhibits Oxaliplatin-induced mechanical allodynia

Testing was performed 7-day after a single challenge with oxaliplatin (2.5 mg/kg, i.v., dissolved in normal saline)
*, p<0.05 vs respective vehicle-treated group (Student’s t-test).
TRPA1 antagonists are active in multiple pain models
AP-00750 inhibits mechanical allodynia after CCI

Experiments were performed 10 days after the lesion.
Drugs were administered at the indicated dose by oral gavage.
TRPA1 antagonists are active in multiple pain models
AP-00750 inhibits mechanical allodynia after CFA

Testing was performed 1-day after a single intraplantar challenge with CFA
*, p<0.05 vs respective vehicle-treated group (Student’s t-test).
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TRPA1 antagonists – first generation
Xanthine derivatives and analogues – generally poorly soluble and poor PK

Hydra

- **HC030031**
- 2007, 5μM
- 2010, 4nM

Hydra/Cubist

- 2013, 93nM
- 2014, <25nM

Glenmark

- 2009, <40nM
- 2010, <50nM
TRPA1 antagonists – first generation
Xanthine analogues with amide isostere - Amgen

rTRPA1 71nM
hTRPA1 131nM

iv Cl = 2.5 L/h/Kg
iv Vss = 1.7 L/Kg
po F = 60%
po T<sub>1/2</sub> = 2.8hr

Assay in AITC-induced flinching in rats

Schenkel, JMC, 2016
**TRPA1 antagonists – second generation**

New generation of smaller TRPA1 antagonists

- **Hydra/Cubist, 2013, 93nM**
- **Janssen, 2010, 7nM**
- **Roche, 2014, 12nM**
- **Roche, 2014, 13nM**
- **Orion, 2012, 15μM**
TRPA1 antagonists – more diverse antagonists?

New generation of smaller TRPA1 antagonists

Orion, 2014, 100nM
Astra Zeneca, 2012, 60nM
Merck, 2011, 19nM
Astra Zeneca, 2014, 110nM

Genentech, 2016
hTRPA1 110nM
rTRPA1 7.3μM

Novartis, 2014

rTRPA1 85nM
hTRPA1 15nM

Iv Cl = 26 ml/min/Kg
Iv Vss = 2.39 L/Kg
Po F = 39%
D_{50} = 5.5mpk in CFA rat

Mustard-induced allodynia in mice
Binding Site Hypothesis 1

Janssen, Amgen, Glenmark, Hydra antagonists

Large Hydrophobic pocket in N-terminal domain
Occupation induces allosteric regulation of TRPA1
Single Occupation $\rightarrow$ ACTIVATES

Site prefers lipophilic side chains

hTRPA1 – C621

JACS, 2015, 137 (50) 15859 JT activator
Mol Pharm, 2013, 83, 1120 pBQN
Binding Site Hypothesis 1

Janssen, Amgen, Glenmark, Hydra antagonists

Hydrophilic pocket with a number of potential contacts. Xanthine core fits well.

hTRPA1 – C621

JACS, 2015, 137 (50) 15859 JT activator
Mol Pharm, 2013, 83, 1120 pBQN
Point mutations identify sites in the pore that play a critical role in AZ-868 and A-967079 binding. Binding of xanthane-like ligands such as HC030031 is not affected by these mutations.

Dabrowski, Biophys J, 2013, 104, 798

Five residues alter affinity for compound 31. No effects on mustard oil activation. Sites mapped onto a homology model of the mouse TRPA1 channel based on KcsA.

Moldenhauser, PLOS, 2014, 9 (9) 1
TRPA1 cryo-EM structure
A Platform to Great Achievements

- Determined at 4Å as 3J9P, released April 2015

HC-030031

Accurately describes cytoplasmic site of covalent activators and some antagonists (BS1); allows classification of different antagonist classes

A-967079

Accurately describes pore region antagonist site (BS2) and confirms binding of A-967079 in this region (but not HC-030031)

Julius, Nature, 2015,
TRPA1 antagonists – hurdles along the way

- Poor DMPK properties
- Low ligand efficiency
- Mixed partial agonist/antagonist properties
- Covalent and non-covalent binding
- Species differences
TRPA1 antagonists – clinical development

And other approaches……

- Cubist/Hydra CB-625 enters clinical development in 2012
  - discontinued for PK reasons

- Glenmark GRC17536 enters clinical development 2010
  - Positive data in patients with PDN announced 2014

- Hydra announces progression of HX-100 into Phase 2 for PDN and asthma January 2016

- Mouse monoclonal antibodies to TRPA1 act as antagonists of multiple modes of channel activation (Gavva et al, J PET, 2014)

Thanks for your attention
Global Platform. One Vision.