New trends in anti-inflammatory drugs

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http://www.lassbio.icb.ufrj.br/
• The inflammatory process: a brief view
• Small Molecules
  ▪ The timeline of anti-inflammatory drugs (AID)
• Targets
  ▪ From COX-1 to kinases
  ▪ Phosphodiesterase-4 (PDE-4) inhibitors
  ▪ Anti-TNFα biopharmaceuticals
• Multi-target drugs: *in-house* results,
  LASSBio-468, new dual DMARD candidate
• Concluding remarks
The inflammatory response can be either acute or chronic, but the local reactions signals are described as the cardinal signs & symptoms of inflammation.
Acute inflammation involves:

- alteration of vascular caliber
  (vasodilation leads to increased blood flow)

- changes of microvasculature
  (increased permeability for plasma protein and cells)

- emigration of leukocytes from microcirculation
  (leukocyte activation leads to elimination of offending agent)

It can be controlled by several AI drugs, including glicocorticoids and NSAI agents
The chronic inflammatory diseases

- Rheumatoid arthritis (RA)
- Inflammatory bowel disease
- Psoriasis
- Alzheimer disease (AND)
- Atherosclerosis
- Stroke / heart attack
- COPD / asthma
- Septic shock
- Cancer
The mediators of the inflammatory process
The inflammatory response is multifactorial!
The arachidonic acid cascade & inflammation

Adapted from Robbins & Cotran's Pathological Basis of Disease, 8th Ed., Kumar et al (eds), Elsevier, Philadelphia (2010)

The enzymatic inhibition promotes the accumulation of the substrate!
Small Molecules
The timeline of AID’s


ASA

Fenamic acid’s

ASA/MoA

Sir John R Vane (1927-2004)

Kendall, Reichstein & Hench

Indomethacin

COX-2

Celecoxib

Multi-target drugs

Cortisone

PK’s*

Krebs & Fischer

TK-i (Imatinib)

* P Cohen, DR Alessi, Kinase drug discovery--what's next in the field?, ACS Chem Biol. 2013, 8, 96
The beginning...

Parke Davis Co.
Claude Winder

ASA

Mefenamic acid

Meclofenamic acid

Ciba-Geigy (Novartis)

Diclofenac

Phenyl acetic class C-linear homologue

1960

1964

1973
From COX-1 to kinases

Merck Co.

From COX-1 to kinases

Merck Co.

T Y Shen
(1924)

sulindac
Clinoril®

non-steroidal anti-inflammatory drug

Sulindac is a prodrug
(1980)

From COX-1 to kinases

Boots Group

![Chemical structure of Ibuprofen *](image1)

Stewart Adams

Ibuprofen *

Six times more active than ibufenac

α-Aryl propionic acid

Profen’s class

C₁-branched homologue

Ibufenac

Syntex (Ian T Harrison)

Naproxen (1976)

Over the counter (OTC) drug


All these initial AI drugs have been discovered under poor understanding of the underlying inflammatory mechanisms!
Targets
From COX-1 to kinases

Pfizer (Searle Co., 1993)

Celecoxib (1998)
The first COX-2 selective nonsteroidal anti-inflammatory drug

COX-2

Discovered in 1991 by Daniel L. Simmons
Brigham Young University

Celecoxib (1998)

Rofecoxib (1999)

Merck Co.

2004 - withdrawn for safety concerns
(use increase the risk of heart attack & stroke)
From COX-1 to kinases

Abbott Laboratories
1996

SRS-A

LTB₄, LTC₄, LTD₄, LTE₄

Zileuton (Zyflo®)
MoA = Inhibitor of 5-lipoxygenase (5-LOX)
Hydroxamic acid scaffold

Merck Co.
1998

Cl

Ultimately achieving blockbuster status

Montelukast (Singulair®)
MoA = cys-leukotriene receptor antagonist (LTRant)
From COX-1 to kinases

“...for describing how reversible phosphorylation works as a switch to activate proteins and regulate various cellular processes.”
The Human Kinome

518 genes PK's
500 k papers
20 k patents
c.a. 10% K

JL Levin, SA Laufer, J Med Chem 2014, 57, 2167
Kinase inhibitors as anti-inflammatory drugs

<table>
<thead>
<tr>
<th>Agent</th>
<th>Targets for therapeutic activity</th>
<th>Indication/Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tofacitinib</td>
<td>JAK3/JAK1/JAK2</td>
<td>RA/Phase III</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Psoriasis/Phase II</td>
</tr>
<tr>
<td>Ruxolitinib</td>
<td>JAK1/JAK2</td>
<td>Psoriasis/Phase II</td>
</tr>
<tr>
<td>VX-509</td>
<td>JAK3</td>
<td>RA/Phase II</td>
</tr>
<tr>
<td>R-348</td>
<td>JAK3</td>
<td>RA/Phase I</td>
</tr>
<tr>
<td>INCB-028050</td>
<td>JAK1/JAK2</td>
<td>RA/Phase II</td>
</tr>
<tr>
<td>Lestaurtinib</td>
<td>FLT3/TrkA/JAK2</td>
<td>AML/Phase III</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Psoriasis/Phase II</td>
</tr>
<tr>
<td>AC-430</td>
<td>JAK2</td>
<td>RA/Phase I</td>
</tr>
</tbody>
</table>

From COX-1 to kinases

Incyte Pharmaceuticals and Novartis

FDA Approval 2011

John J O’Shea
Immunologist from NIH,
described the significance
of JAK3 inhibition as
AI target

JAK1/JAK2-selective inhibitor

\[
\text{IC}_{50} \text{ (nM)}
\]

JAK1 = 6.4
JAK2 = 8.8
JAK3 = 487

RA
Myelofibrosis (progressive)
& plaque psoriasis

Ruxolitinib
\(\text{C}_{17}\text{H}_{18}\text{N}_{6}\)

pyrrole[2,3-\text{d}]pyrimidine

pyrazole
**From COX-1 to kinases**

Tofacitinib * *(CP-690,550)*

JAK1/JAK3-selective inhibitor

IC$_{50}$ (nM)
- JAK1 = 15.0
- JAK2 = 77
- JAK3 = 55

Crystal Structure of CP-690,550 in the JAK3 active site showing hinge binding

* approved by FDA, in 2012, for the treatment of RA and it is in clinical Phase II studies for the treatment of chronic plaque psoriasis (prevention of organ transplant rejection)

Pfizer

European Respiratory Society Annual Congress 2013

In 2015, annual sales of kinases drugs are anticipated to US$20 billion
Phosphodiesterase-4 (PDE-4) inhibitor

Approved by the FDA in March, 2014, for psoriatic arthritis & in September, 2014 for plaque arthritis.

Roflumilast (2010;Daxas®) is a second drug that acts as a selective inhibitor PDE-4 used for COPD control

World's best-selling drugs of all time

Antiinflammatory biopharmaceuticals

Anti-TNF-α drugs
Anti-TNF-\(\alpha\) Biopharmaceuticals

Protein-based anti-TNF-alpha Therapies in Clinical Use*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Status</th>
<th>Biological Form</th>
</tr>
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<tbody>
<tr>
<td>Etanercept</td>
<td>approved</td>
<td>soluble TNFR2 coupled to Fc portion of IgG</td>
</tr>
<tr>
<td>Infliximab</td>
<td>approved</td>
<td>chimeric anti-human TNF antibody</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>approved</td>
<td>anti-human TNF antibody</td>
</tr>
<tr>
<td>ISIS 104838</td>
<td>clinical</td>
<td>TNF anti-sense</td>
</tr>
<tr>
<td>Onercept</td>
<td>clinical</td>
<td>soluble p55 TNFR</td>
</tr>
<tr>
<td>Humicade</td>
<td>clinical</td>
<td>anti-TNF humanised IgG4</td>
</tr>
</tbody>
</table>


* protein-based injectable anti-TNF\(\alpha\) therapies
Could be effective a single target drug in the treatment of multifactorial diseases?
The treatment of multifactorial diseases (e.g. inflammatory chronic diseases), with drugs designed for a single therapeutic target, will always palliative!

These disorders require multi-target drugs as dual agents.

**A simple drug don't work well in complex diseases**
Multi-target drug: in-house results

LASSBio-468, new dual candidate

New dual anti-inflammatory lead drug-candidate

- Phosphodiesterase inhibitor (PDE-4i)
  & TNF-α modulator activity
TNF-α (Tumor necrosis factor-alpha)

TNF-α is a pleiotropic cytokine with important pro-inflammatory functions

The first pharmacophoric scaffold

2-(2,6-Dioxo-3-piperidinyl)-1H-isoindole-1,3(2H)-dione

Thalidomide (THLD)
Oral TNF-α inhibitor

Advantages of small molecule
Convenient non-injectable
Facilitate tissue penetration
Possible once a day dosing
Reduced immunosupression
Easier synthesis lower costs

V Richmond et al., Small Molecules as Anti-TNF Drugs, Curr Med Chem. 2015, 22, 2920
The Second Target Election: PDE

The pathogenesis of inflammation
Phosphodiesterase-4 is predominant in inflammatory cells

PDE = Phosphodiesterase

From: MA Palladino et al., *Nat Rev.Drug Discov* 2003, 2, 736
Chemical Intuition
The molecular design of new dual agent: anti-TNFα & PDE-4i

Molecular hybridization

phthalimide

sulfonamide

lipophylic

isosteres

Congeneric series

Molecular diversity

Montana et al., 1998

Medicinal chemist dream....
The molecular design of new dual agent: anti-TNFα & PDE-4i

Molecular hybridization

phthalimide

sulphonamide

Drug Design

Montana et al., 1998

Molecular Hybridization: A useful tool in the design of new drug prototypes, Curr Med Chem 2007, 14, 1829
Congeneric series

LASSBio-449

LASSBio-468

LASSBio-469

LASSBio-470

Bioisosterism

LASSBio-544


Synthesis of congeneric series

phthalic anhydride

C₁₂H₈O₃

120°C
1 hr
2.0 M

1.0 M

CISO₃H

0°C to rt, then 60°C
1 hr

Overall yield: ca. 25%

~ 0.5 M, 200 g

LASSBio-544
69%

LASSBio-469
58%

LASSBio-449
65%

LASSBio-470
67%

LASSBio-468
72%

C₁₈H₁₆N₂O₄S₂
M.W. = 388.45

Effect of new compounds and thalidomide on neutrophils influx, induced by LPS into BALB/c of mice lungs (10 mg/kg, DMSO; ip)

Results are expressed as means ± SEM of seven animals.
Effect of compound LASSBio 468 (50 mg/kg, ip) on TNF-\(\alpha\) levels and neutrophils influx (BALB/c; lung exudate)

Inhibition of the production of TNF-\(\alpha\) promote the elevation of intracellular levels of cyclic 3',5'-adenosine monophosphate (cAMP) in leukocytes, associated with inhibition of PDE-4 activity.*

* DO Procopio, MM Teixeira, MM Camargo, LR Travassos, MA Ferguson, IC Almeida, RT Gazzinelli, Differential inhibitory mechanism of cyclic AMP on TNF-\(\alpha\) and IL-12 synthesis by macrophages exposed to microbial stimuli. *Br. J. Pharmacol.* **1999**, *127*, 1195
TNF-α ED₅₀ 2.5 mg/Kg

PDE-4 inhibitor

C₁₈H₁₆N₂O₄S₂

Dr Claire Lugnier (CAPES-COFECUB; LASSBio-Strasbourg)
Université Louis Pasteur, Strasbourg, FR.
Laboratoire de Pharmacologie et de Physicochimie des Interactions Cellulaires et Moléculaires.

IC₅₀ = 30.1 µM
cf. PDE-1, 2, 3, >> 150 µM;

Effect of the treatment with LASSBio-468 (50 mg/kg po) on hind paw edema in adjuvant-induced arthritis (AiA-model) (*Mycobacterium tuberculosis*) in rats

Dr Magna Suzana Alexandre-Moreira  
LASSBio, Universidade Federal do Rio de Janeiro
Histopathological results

(1) Photomicrography of granulomatous hepatitis in the control animals (HE – 100X);
(2) Animals treated with thalidomide (HE – 100X);
(3) Animals treated with LASSBio 468 (HE – 200X);

* A positive control was performed *M. tuberculosis*.

**LASSBio-468 has a protective effect on inflammation development mediated by immunomodulatory macrophage activity**

Christina M Takyia
ICB, Universidade Federal do Rio de Janeiro
LASSBio-468

A new DMARD lead-candidate

LASSBio-468 is a new dual anti-inflammatory agent (DMARD), active at TNF-α production with inhibitory activity on PDE-4. This new achiral compound is an immunomodulator lead, without proliferative activity in the concavalin-A mitogen assay, in contrast to TLDH. It is an useful lead to therapy of rheumatoid arthritis & septic shock syndrome.

“... when it comes to drug discovery you’re not trying to make complicated molecules, but make molecules that will be effective ... “

Barry J. Price
Research Director Glaxo (1967-1995)
Concluding remarks

• Inflammation is so broad that, there remains both need and opportunity for new, distinctive, and successful small molecule agents, including selective multitarget candidates.

• Several recent potential new targets for AI drugs were identified as \( m \)-PGES-1, \( c \)PLA2a, LTA\(_4\)-hydrolase, from eicosanoids class; from kinases are MK2, Sik kinase, Janus kinases (JAKS), IKK\( \beta \), Bruton’s tyrosin kinase (Btk), p38 MAPK inhibitors. GPCR’s also represents an important pathway to develop new AI agents acting as CCR1, CCR2 & CB2 agonists.

• The discovery of the integral role of the Inflammasome in driving inflammatory processes, has now led to efforts to directly block its formation and actions and represents an important pathway to control inflammatory disorders, including chronic ones.
“...medicinal chemists today live in exciting times... their work can have a beneficial effect on millions of suffering patients – surely an important motivating factor for any scientist...”

Joseph G. Lombardino & John A. Lowe, III

The Role of the Medicinal Chemist in Drug Discovery – Then and Now,

Acknowledgements

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