Whole cell analytical assay for targeting purine and pyrimidine biosynthesis: Application for discovery of new anti-proliferative immunosuppressive drugs

Larissa Balakireva, Vanessa Croset, Nicolas Godard
NovoCIB, 115 avenue Lacassagne 69003 Lyon, France

www.novocib.com

ABSTRACT

The depletion of T- and B-lymphocytes due to genetic disorders of purine and pyrimidine metabolism highlights the importance of these pathways to immune response. The therapeutic potential of targeting enzymes of the nucleotide biosynthesis was displayed by successful application of anti-metabolite drugs in the treatment of porosiasis, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA, e.g. Arava® - leflunomide, inhibitor of dihydroorotase dehydrogenase (DHODH)) and to prevent transplant rejection (Cellcept® - a prodrug of mycophenolic acid MPA, inhibitor of inosine monophosphate dehydrogenase (IMPDH).)

i. We have cloned a human recombinant IMPDH II and developed in vitro enzymatic assays for search of new IMPDH inhibitors.

ii. We have developed a whole cell assay to select compounds that are able to penetrate cells and target the intracellular IMPDH.

iii. We have developed whole cell analytical assays, based on separation, identification and quantification by HPLC of complete spectra of nucleosides and nucleotides to study the effect of drugs on nucleoside metabolism.

Whole cell analytical assay for nucleotide biosynthesis inhibition

In order to:

• confirm the ability of IMPDH inhibitors in vitro selected to decrease the cellular pool of GTP;

• estimate the intracellular IC50;

• evaluate the compounds specificity by comparing GTP, ATP, UTP and CTP cell contents; we have developed a whole cell bio-analytical assay that consists in separating and quantifying nucleotides extracted from drug treated cells.

The cell assay has been validated using known inhibitors of purine and pyrimidine biosynthesis: MPA, which targets IMP dehydrogenase, and leflunomide (LFM), which acts on dihydroorotate dehydrogenase (DHODH). According to previously published data, MPA inhibits the formation of guanosine nucleotides, whereas LFM decreases the level of uridine nucleotides.

IMPDH, a choice target for the treatment of immunological diseases

Inosine Monophosphate Dehydrogenase (IMPDH) converts inosine 5'-monophosphate (IMP) to xanthosine 5'-monophosphate (XMP) using NAD+ as a cofactor.

The oxidation of IMP to XMP is considered as the pivotal step in the biosynthesis of guanine nucleotides[1] whose decrease resulting from IMPDH inhibition interrupts the nucleic acid synthesis in proliferating cells. IMPDH Type II is the predominant isoenzyme of the enzyme and is selectively expressed in lymphocytes and tumor cells[1].

IMPDH is considered as a potent target for cancer chemotherapy (e.g. AVN-944) and for the treatment of autoimmune diseases such as psoriasis, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Cellcept® - a mycophenolic acid (MPA) prodrug, is a successful immunosuppressive agent (CHA 86 in 2006) used to prevent transplant rejection. The extension of Cellcept® therapeutic applications (orphan drug for Mysisstheria gravis, phase III for SLE) makes IMPDH an attractive validated target to treat numerous immunological diseases.

Besides MPA, analogues and nucleoside analogues (ribavirin - Virazole®, Rebetol®, Colepyrazole - mitozorine® - Bredinin® and tiazofurin - Tiazole®), other NCEs have been identified as IMPDH inhibitors[2,4,5] and entered development trials.

The IMPDH II atomic structure has been resolved and it provides a valuable background for further lead optimization.

All this demonstrates how promising the research of new IMPDH inhibitors is and why compounds are worth being evaluated on such a highly pertinent target.

Enzymatic characterization of human IMPDH II

We have established and validated:

1. an in vitro enzymatic assay for the identification of new IMPDH II inhibitors

2. a whole cell based assay to select compounds that are able to penetrate cells and reach intracellular IMPDH.

3. a bio-analytical method for the search of inhibitors of the purine and pyrimidine biosynthesis, which is considered as a rich source of targets for immunosuppression and anti-cancer treatment.

References


Fig. 1: Chemotherapeutic potential of IMPDH inhibitors for targeting purine and pyrimidine biosynthesis: Application for discovery of new anti-proliferative immunosuppressive drugs

Fig. 2: Enzymatic characterization of human IMPDH II

Fig. 3: ImpDH inhibition by MPA IC50 = 7.8µM at 25°C

Fig. 4: Effect of IMPDH inhibitors (MPA, mizoribine) and DHODH inhibitor (LFM) on intracellular content of GTP and CTP. The nucleotide extraction, separation and quantification were done as described above.

Conclusions

We have established and validated:

1. an in vitro enzymatic assay for the identification of new IMPDH II inhibitors

2. a whole cell based assay to select compounds that are able to penetrate cells and reach intracellular IMPDH.

3. a bio-analytical method for the search of inhibitors of the purine and pyrimidine biosynthesis, which is considered as a rich source of targets for immunosuppression and anti-cancer treatment.

Fig. 5: Whole cell analytical assay for nucleotide biosynthesis inhibition

Fig. 6: In vitro enzymatic assay for the identification of new IMPDH II inhibitors

Fig. 7: Chromatograms of TCA extracts of cultured HuH7 cells treated with increasing concentrations of MPA for 48h. The nucleosides contents of cells extracts were analyzed using HPLC Agilent 1100 fitted up with zorbax-C18 column. The nucleosides are separated using a Zorbax EclipsePlus C18 column. A: focus on GTP and IMP B: focus on UTP and CTP.