BUDAPESTI MŰSZAKI ÉS GAZDASÁGTUDOMÁNYI EGYETEM
VEGYÉSZMÉRNÖKI ÉS BIOMÉRNÖKI KAR

Novel Small-Molecule Inhibitors of Phosphodiesterase 4 Isoenzyme and Human Leukocyte Elastase in the Treatment of Airway Diseases

Tézisfüzet

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INTRODUCTION

Asthma and chronic obstructive pulmonary disease (COPD) are common, severe inflammatory diseases of the respiratory tract for which there is no efficient medical treatment. Current therapy for asthma is for the most part effective and comprises bronchodilators and anti-inflammatory glucocorticoids. In contrast, pharmacotherapy of COPD is much less effective. In addition, there are potentially undesirable side effects of current treatment of both conditions. Consequently, there are legitimate reasons for searching alternative or additional therapies for asthma and COPD.

COPD and asthma is a worldwide public health problem that reduces the quality of life, increases the frequency of contact with health care providers, causes frequent hospital admissions and carries an increased risk of premature death for those affected. These chronic airways diseases such as asthma, COPD, and allergic rhinitis are a huge source of morbidity and mortality worldwide, and unlike most other categories of disease, which are decreasing in prevalence, chronic airways diseases are on the increase. There is a great need for more and better care of these diseases.

Finally there is an important unmet medical need in the treatment of asthma and inflammation. We selected two important targets which can take an important role in the development of inflammatory part of COPD and asthma. Selected targets are Phosphodiesterase 4 isoenzyme (PDE4) and human neutrophil elastase (HLE).

The goal of the study was to develop orally active and selective PDE4 and HLE inhibitors for the treatment of COPD and asthma.

SCIENTIFIC BACKGROUND

We developed and set up a drug research system within the company in which we use the in vitro, ex vivo, in vivo models together in order to evaluate the effect of new drugs in order to accelerate the drug development process.

Interest in PDE4 as a molecular target for new antiasthmatic and anti-inflammatory drugs has increased greatly over the past years. This interest is supported by several factors. Observation that PDE4 is the dominant cAMP hydrolyzing activity in immune and inflammatory cells. Prototypical PDE4 inhibitors such as rolipram suppress the activation of these cells. Rolipram and other first generation PDE4 inhibitors produce marked anti-inflammatory actions in animal models. Unfortunately the use of these compounds was limited by serious side effects. The side effects observed include increased gastric acid secretion, nausea and vomiting. So, the challenge to our drug discovery efforts has been the design of novel PDE4 inhibitors that maintain the anti-inflammatory actions of rolipram with a reduced potential to elicit side effects.

The protease/anti-protease inbalance is still considered to be a major pathogenic determinant in COPD [165, 180]. The imbalance between HLE and endogenous antiproteases, when it happens, may cause various inflammatory responses. These include epithelial damage, increased microvascular permeability followed by mucus hypersecrtion, induction of bronchial secretory cell metaplasia and mucociliary disfunction. Excessive HLE shows a profound destructive profile, destroys the normal pulmonary structure followed by the irreversible enlargement of the respiratory airspaces as seen mainly in emphysema, while inducing increased microvascular permeability followed by mucus hypersecretion as seen mainly in chronic bronchitis.

Goal of the research was to find orally active PDE4 and HLE inhibitors in the treatment of asthma and COPD and other indications where the inflammation is involved in the pathogenesis of diseases.

EXPERIMENTAL

A new and informative, test system containing in vitro, ex vivo and in vivo models for the search of orally active PDE4 and HLE inhibitors was developed.

Cell based and ex vivo assays are more complicated system than an in vitro assays, these are a very good transition between a simple in vitro and a complicated, but very predictive in vivo system including animal models which mimic the human disease conditions.

In the PDE4 project an important task was the good prediction of side effect profile of the new compounds. For this purpose we set up an in vitro rolipram binding experiment on human brain together with an ex vivo rolipram binding experiments on mice. The in vitro assay predicted the affinity of compound to HPDE4 site, the ex vivo assay predicted the penetration of compounds into the central nervous system. As we showed out, the results of ex vivo binding assay correlate well with behavioral effects (inhibition of spontaneous motility in mice) of investigated compounds, and the results give a good prediction for side effect profile.

We had very important assays for the investigation of bronchodilating effect of PDE4 inhibitors on tracheal preparation in vitro and in vivo on guinea pig, the inhibitory effects of compounds on histamine and ovalbumin induced bronchoconstriction.

The anti-inflammatory activity of compounds was predicted in vivo in guinea pig and mice. The ovalbumin induced cell migration in guinea pig and the LPS induced TNFalpha release in mice. In The experiments the migrated cells (total cell number and eosinophils, neutrophils) were measured BAL of guinea pigs and the TNFalpha release was measured in mice plasma.

The side effects were also examined on dog, Suncus murinus and mice. The emetic activity was examined on dog and Suncus murinus and the CNS, behavioral side effects on mice. The therapeutic ratio of the PDE4 inhibitors were calculated on the basis of ED50 values on different animal models.

In the HLE project, we set up an in vitro kinetic assay (slow tight binding type inhibition) using small molecular weight substrate. We set up other in vitro enzyme assay, inhibition of enzyme activity using natural substrate, elastin, and examination of activity of compounds

on free, membrane bound and intracellular elastase, using human neutrophils in these assay systems.

We developed a very important ex vivo assay in mice, for the assessment of oral activity of HLE inhibitors.

The in vivo activity of HLE inhibitors were investigated mice using acute lung hemorrhage model induced by HLE, two types paw oedema models, where the paw oedema was induced by HLE and carrageenan. We examined the dose dependence and the duration of action of inhibitors.

The non airway disease related pharmacological effect of HLE inhibitors were also investigated in vivo, on splanchnic artery occlusion/reperfusion model, TNBS induced colitis model in mice and on coronary ischemia-reperfusion model in anaesthetized rabbits.

With the help of these in vivo models we obtained a very good pharmacological profile from the investigated HLE inhibitors.

RESULTS

We demonstrated the PDE4 isoenzyme selectivity of drotaverine. Because drotaverine has a selective PDE4 inhibitory effect without inhibiting PDE3 and PDE5, in addition to this observation it has a significant calcium antagonist potential, these biological activity may give a correct explanation for the slight cardiovascular side effects of drotaverine. Drotaverine has been launched several years ago and PDE4 inhibitors related side effects have not been observed under the medical treatment of drotaverine.

On the basis of results of drotaverine we developed a new isoquinoline type PDE4 inhibitor, SSR161052 as a candidate for development compound as a powerful PDE4 inhibitor with potent anti-inflammatory and bronchorelaxant activity without important side effects on the basis of in vitro and in vivo investigations.

SSR161052 is a selective and competitive inhibitor of PDE4, its selectivity is higher than 2 log. The compound has a nanomolar potency on PDE4, IC50 = 3 nM. It is essentially inactive on PDEs, 1, -2, -3 and -5. SSR161052 has a good selectivity on PDE4 inhibitory activity versus rolipram binding site and HPDE4 activity versus LPDE4 activity suggests an improved therapeutic ratio.

SSR161052 has an important activity on whole cell system, pre-incubating the cells (A549 and HL60) with SSR161052 results a significant increase in the intracellular cAMP concentration indicating a good cell penetration, which is a very important properties of phospodiesterase inhibitors.

SSR161052 induces concentration-dependent relaxation on spontaneous tracheal tone of intact tracheal preparation. Its effect is significant and it is ten times stronger , than that of SB207499. The calculated relaxant EC_{50} value is 85 nM. This value is a bit higher than the PDE4 inhibitory IC_{50} value (3 nM). This concentration (85 nM) is enough to increase the intracellular cAMP level in A549 and HL60 cells. So it is concluded that the PDE4 inhibitory effect of SSR161052 is responsible for the tracheal relaxant effect.

SSR161052 has been shown to exhibit anti-inflammatory and bronchodilator properties. SSR161052 dose dependently attenuates histamine and allergen – induced bronchoconstiction in sensitized guinea pigs (ED50 = 0.38 mg/kg against histamine and ED50 = 0.026 mg/kg against ovalbumin). SSR161052 is very potent in attenuating allergen induced-bronchospasm. Parallel with this activity SSR161052 has a significant anti-inflammatory activity. The potential of compounds as anti-inflammatory agents were assessed on the basis of their ability to inhibit the TNF_{α} release from murine macrophages in vitro and to reduce murine serum TNF_{α} levels after LPS injection in vivo. These studies indicate that SSR161052 is not only very active drug on airway inflammation, but it is very potent in attenuating allergen induced bronchospasm too, acts as an effective bronchodilator. All activity is related to its PDE4 inhibitory potency.

To determine the in vivo therapeutic window of SSR161052, the side-effects were investigated in parallel with its anti-inflammatory activity. All investigated PDE4 inhibitors decreased the spontaneous motility in mice, but in a different dose-range. Effects of

compounds on spontaneous motility in mice predict rather well their CNS side effects. Rolipram, SB-207499 and LAS-31025 decreased LPS-induced TNF_α level in mice in similar dose range where these compounds inhibit spontaneous motility in mice. We could not observe important target selectivity in vivo. The selectivity of these compounds is rather low in vivo. Drotaverine and our candidate for development SSR161052 decreased serum TNF_α level after LPS injection at doses approx. twenty times lower than those which induced CNS side-effect. The in vivo selectivity of SSR161052 and drotaverine is outstanding (the exact value is 17). The therapeutic ratio is more pronounced if we compare the CNS side effect and inhibitory potency of SSR161052 on OVA induced bronchoconstriction in guinea pig (the value is 450). PDE4 inhibitors induce emesis in Suncus. murinus as well as in dogs and ferrets. We used dog and Suncus murinus for the assessment emetic activity of drugs on human. SSR161052 has emetic activity, maximal emetic effect was observed at 1 mg/kg i.v. in dogs and 30 mg/kg in Suncus murinus after p.o., but SSR161052 exerts its beneficial activity in much lower dose range, ED_{50} values are 0.026 mg/kg against ovalbumin induced bronchconstiction in guinea pig and 0.69 mg/kg on LPS induced TNF_α release in mice. Finally SB-207499 and SSR161052 have similar emetic activity, but SSR161052 has a much better therapeutic ratio than SB-207499.

Finally we could conclude that SSR161052 is a very active and selective, orally active PDE4 inhibitor. It has a potent spasmolytic and anti-inflammatory activity and on the basis of in vivo experiments we could predict an improved therapeutic ratio compared to reference compounds (e.g. SB-207499).

SSR69071is a saccharin derivative with a pyrido-pyrimidine moiety, specially designed molecule, sensitive to nucleophilic attack. SSR69071 is a potent, competitive and slow tight binding type inhibitor of HLE (Ki = 0.015 nM). SSR69071 has a faster association rate and slower dissociation rate than ZD-8321 leading to a more stable HLE - Inhibitor complex. SSR69071 possesses higher potency for human than for mice and rat elastase. SSR69071 has marked specificity for HLE versus a large range of receptors and enzymes. Elastase exists either as free enzyme (released from activated leukocytes) or as leukocyte intracellular and surface bound enzyme (resistant to inhibition by natural inhibitors). In our

experimental conditions, SSR69071 inhibited both free and surface bound HLE. The HLE inhibitory activity of BAL (measured in vitro) increases significantly obtained from mice orally treated with SSR69071. In this model, SSR69071 has a dose-dependent and long-lasting (at least four hours) activity. Administered either i.v. or p.o, SSR69071 inhibits tissue injury (lung haemorrhage in mice) triggered by exposure to HLE. In addition, given by the i.v. or oral routes, SSR69071 inhibits tissue injury and inflammatory reaction (neutrophils migration) triggered by splanchnic artery occlusion/reperfusion in mice. SSR69071 administered p.o., prevents the inflammatory reaction triggered by HLE or carrageenan (paw oedema) in rats**.**

In a variety of the experimental settings indicated above, SSR69071 has a long duration of pharmacological activity. SSR69071 shows a tendency to increase its pharmacological activity after repeated administration over a period of 7 days.

SSR69071 has a poor microsomal stability in rodents but good stability in human plasma and human microsome and hepatocyte preparations. SSR69071 is well absorbed in the CACO2 model and its bioavailability is between 12 - 30 % in mice, rats and dogs. We used a number of models in mice and we observed a rather long duration of action of SSR69071 on these models There is an apparent contradiction between the long duration of action and the stability data. The slow tight binding kinetic nature of HLE inhibition by SSR69071 may resolve this contradiction. Slow, tight binding inhibitors are important from a pharmacological point of view, because the compound once bonds to its target it inhibits the enzyme function even after the free drug has been cleared from the circulation or from the site of action.

SSR69071 is a selective, specific and very potent inhibitor of HLE. SSR69071 is orally active and shows good target tissue penetration (bronchoalveolar lavage fluid) in mice. Pharmacokinetic studies indicate acceptable bioavailability of the compound. However, SSR69071 may have a higher potency and longer duration of action than ZD-8321 (reference compound) in the human setting as a result of the stability of the HLE-SSR complex (slow dissociation rate) and its longer duration of action shown in animal models.

THESIS

1, We demonstrated the PDE4 isoenzyme selectivity of drotaverine. Because drotaverine has a selective PDE4 inhibitory effect without inhibiting PDE3 and PDE5, in addition to this observation it has a significant calcium antagonist potential, these biological activity may give a correct explanation for the slight cardiovascular side effects of drotaverine. Drotaverine has been launched several years ago and PDE4 inhibitors related side effects have not been observed under the medical treatment of drotaverine.

2, We developed and set up a new drug research system, in which we use the in vitro, ex vivo, in vivo methods together in order to evaluate the effect of new drugs in order to accelerate the drug development process. I describe here the development process of an orally active PDE4 and HLE inhibitors, using this process finally we developed and selected PDE4 and HLE inhibitors for precilinal phase.

3, We set up an in vitro and in vivo test system for the prediction of potential side effect profile of PDE4 inhibitors.

4, We developed a new ex vivo test for the assessment of oral activity of HLE inhibitors on mice. Ex vivo activity of HLE inhibitors were detected from BAL from mice.

5, We developed acute lung haemorrhage model induced by intratracheally administered HLE in mice for the prediction of in vivo oral activity of HLE inhibitors. Advantages of this model compared to hamster model are: less amount of compound is needed, results are more reproducible and hamsters have unique metabolic route for some chemical moiety.

6, We developed a new isoquinoline type, selective PDE4 inhibitor with improved side effect profile. This type of PDE4 inhibitors have both antiinflammatory and bronchodilator properties. Oral activity, long duration of action and more pronounced effect after repetitive

treatment support the advantages of this type of isoquinoline type of PDE4 inhibitors further.

7, We developed a new type saccharin derivative human leukocyte elastase inhibitor. This compound, SSR69071 is structurally unrelated to any competitor in this field. This is a competitive and slow tight binding type inhibitor of HLE with Ki value in picomolar range (Ki = 0.015 nM). This inhibitory kinetic profile leads to an extremely stable enzyme inhibitor complex, with 62 hours half life. This very stable enzyme inhibitor complex leads to the extremely potent in vivo activity.

8, We proved an important advantage of slow, tight binding type enzyme inhibitory kinetic. Slow, tight binding inhibitors are important from a pharmacological point of view, because the compound once bonds to its target it inhibits the enzyme function even after the free drug has been cleared from the circulation or from the site of action.

PRACTICAL APPLICATION

SSR161052 was selected as a candidate for development compound, but unfortunately the result of a two weeks toxicology study on mice and rabbit prevented the entering into the preclinical phase. SSR161052 caused a very serious gastrointestinal toxicity on mice and rabbits.

SSR69071 entered into preclinical development in March 2000. Phase I clinical trial was planned to start in 1Q2003, when the preclinical package would have been completed. No major, unresolvable issues were recorded during the preclinical development phase until July 2002 when positive results were reported in two in vitro genotoxicity assays. Further genotoxicity tests were then also performed and these investigations also supported the genotoxicity of SSR69071. On the basis of these unwanted effects, the development of SSR69071 was stopped during the preclinical phase.

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