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A Mystery of the Inhibitors of Human Neutrophil Elastase. Why they are mosty not active in clinical trials and could the system of autoimmune response to human elastin be responsible for that?

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Abstract

Degradation of the extracellular matrix (ECM) is part of the pathology process of many desises. Human neutrophil elastase (HNE) is responsible for degradation of the elastin and formation of the elastin fragments, well known mediators of inflammation and inducer of ant elastin antibodies synthesis. Therefore, HNE is a subject of intense investigation in drug design and clinical studies to prevent elastase-mediated pathology. More than 15 clinical studies were reported in literature without success and only ONO-5046, HNE inhibitor has been approved for clinical use in Japan and Korea Republic, but not in USA. Any type of inhibitor, always bear structural similarity to the natural substrate of the enzyme. They occupied this same region of the enzyme. For HNE natural substrate is the elastin, therefore antibody to elastin, have to bind inhibitors of HNE also. This resulted in „neutralisation" of inhibitors by removing them from place of their action and lack of activity of HNE inhibitors in the clinical trials. It could be a general rule concerning all the enzymes degradation ECM (extracellular matrix) as before was noticed in case of collagens. However, no attempts to the explanation were presented.

In 1968 Janoff and Scherer, first reported that human neutrophil elastase (at this time called polymorphonuclear leukocyte elastase) is a main mediator of inflammation, induced by the human neutrophils [1]. Today it is clear that uncontrolled activity of human neutrophil elastase is one of the main factors driving pathological proceses during a large number of desises. Therefore, the design and synthsis of inhibitor human neutrophil elastase are active project at many academic and Industrial institutions [2]. Recently, use of the inhibitors HNE become interesting approach to the anticancer treatment also [3]. Inhibitors of HNE are intensively investigated in clinical trias and at least 15 of that are reported in literarure. Only two of such substances are approved for the use in human patient. A first one is a Prolactin (human, natural alpha-1-proteinase Inhibitor alpha-1-PI, isolated from human blood plasma) for augmentation and maintenance therapy in individuals with alpha-1-proteinase inhibitor genetic deficiency and clinical evidence of emphysema. It is not a typical, small molecular weight drug and its use is just to correct the level of alpha-1-PI. The second one is sivalestat (ONO-5046 from ONO Pharmaceutical) nonpeptidic inhibitor, approved for clinical use in Japan and Korea Republic but not In USA [4].

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Ono-5046, N-{2-[4-(2,3-Dimethylpropionyloxy) phenylsulfonyl amino)] benzoyl} aminoacetic acid, is a competitive inhibitor of the HNE with Ki= 200 nM. It has unique strcture, not similar to the natural substrate of the enzyme where elastin posess several times repeted fragments of Val-Gly-Val-Ala-Pro-Gly [5]. Fragments of extracellular matrix are known to be an inflammatory mediator and frequently induce an antibody synthesis also [6]. Clearly, the HNE recognised such fragments of elastin and proteolytically hydrolysed peptide bound at Valine-carboxylamide residue. The methoxy-succinyl- Ala-Ala-Pro-Val-(p-nitroanilide) is an excellent chronogeny substrate of HNE, frequntly used in the kinetic investigation of enzyme inhibition [7]. Process of design the structure of inhibitors for serine protease (and in fact for any enzymes) start with the analysis of the structure of best substrate for the enzyme. Sometimes, a small change in the structure of best substrate molecules could provide us a quite good inhibitor for given enzyme. In another words, for the inhibitor of HNE, its structure has to contain structural fragment similar as much as possible, to the structure of Val-Pro-Val in the region responsible for its binding. The HNE posess a pocket, perfectly profiled to bind this unit which we well know from the investigation the structure of best substrate. These elements of the substrate structure, decide about the specificity of the enzyme also.

All inhibitors of the HNE unsuccessfully investigated in clinical studies contain such fragment. Sometimes is not easy to see that at flat 2D driwing, but 3D picture clerly could these visualized. Very instractive here, is the example of the beta-laktam antibiotics, wchich are mimetics of acyl-D-alanyl-D-alanine, as first reported by Tipper and Strominger [8]. Antielastin antibodies, policlonal in nature, bind a veriety of fragments of elastin and just by an accident could bind that one, which is recognised by the substrate binding pocket of HNE also. Because of this structural similarity, the elastin antibodies should bind also the inhibitors of HNE and removed them from the side of pathological process and decrease their effective concentration. As results, we will see that clinical trials at Phase III will not show the Pharmacology efficacy. Recently a new generation of non-peptidyl inhibitors of HNE, on the base of dihydropyridine scaffold were reported [12,13]. They are very potent inhibitors of HNE with Ki in picomolar range and designed to inhibit Proteinase 3 simultaneously [13]. Such dihydropyridine scaffold are used to build up the mimetic of bioactive conformation of substrate for HNE and introduce this fragment to the structure of designed inhibitor. We can only hope that such bioactive conformation is not similar (or identical) to conformation of elastin in the region of binding of HNE. In such case inhibitors will be bind effectively by the elastin antibodies before they have a chance to inhibit HNE in vivo (and proteinase 3). In fact, it could be a general phenomenon concerning all enzymes degradating extracellular matrix, where its fragments induce the synthesis of antibodies [6]. Over twenty years ago, Greenwald pointed out similar problem with the matrix metalloproteinases (MMP's) inhibitors which, even today, after more that forty years of investigation in clinic, did not resulted in a new drug [14]. MMP's are responsible for the collagen's degradation. Collagen fragments, are immunogenic [6], and probably in vivo the collagen antibodies, are able to bind the inhibitors of MMP's also.

In 1996 Paul suggest that catalytic antibodies may be a fairly routine component of natural immune response [15]. They are frequently discovered in normal and pathological conditions [16]. The crystal structure of active hydrolytic antibodies with a phosphonate-type transition state analog (hapten) bound to the active site has been resolved [17]. This antibody active site contain a Ser-His dyad structure insted of the Ser-His-Asp catalytic triad of serine protease. We do not know if synthesis of such type of ant elastin antibodies take place in vivo and no one reported that yet. If this really occur, it will complicate picture even more, mainly because the possibility of neutralisation of natural inhibitors of proteases as alpha-1-proteinase and alpha-2-macroglobulin and others, small molecular weight proteins that are inhibitory for serine proteases [18]. If these take place in vivo, need to be investigated.

It seems, we have a kinetic trap, leading to the neutralisation of HNE inhibitors, set up by the structural chemistry (which in fact is an autoimmune response). There is not an obvious solution. However, we could try to establish a new assay to measure binding of the inhibitors HNE to the human ant elastin antibodies and tested in such system all inhibitors of HNE which we have in ours lirbries of compounds. As a standard we should use a Sivelestat (ONO-5046), the only HNE inhibitor, which reach the market. ONO-5046 probably does not bind (or very weakly bind) to the human elastin antibodies. (a problem here, could be an evolution of antibodies undergoing during immune responded?). A new, effective in vivo inhibitor of HNE need to have an identical profile – does not bind to elastin antibodies and being a good inhibitor of HNE. These searches could bring us a new lead structure to a new type, of an effective in vivo inhibitor of HNE. Alternatively, we need to establish a new way to design inhibitors, which could omited the limitation forrmed by structural chemistry/ autoimmune response to the human elastin. (If it is possible?).

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