Biochemical Approaches to Drug Targeting

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Potential therapeutic exploitation of the pulmonary polyamine uptake system

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Introduction

In this brief communication, we describe the existence of a pulmonary polyamine uptake system. We discuss the presence of similar uptake systems in a number of other cell types, in particular certain tumour cells. Finally, we discuss the possible therapeutic exploitation of this pulmonary polyamine uptake system, in particular, in relation to the design of novel anticancer agents and better radioprotective agents and finally its possible exploitation in antiprotozoal chemotherapy.

The discovery of a pulmonary polyamine uptake system

In the course of studies in one of our laboratories on the mechanism of pulmonary toxicity of the herbicide paraquat [1,1′-dimethyl-4,4′-bipyridilium], we noted that a critical component of the mechanism was the initial accumulation of paraquat in the lung [1]. This uptake was shown to occur by an active energy-dependent transport system [2]. Endogenous polyamines (putrescine, spermidine and spermine) appeared to be the natural substrates for this uptake system [3]. The polyamines were also accumulated in the lung by an active energy-dependent uptake system [3, 4], and they competitively inhibited the accumulation of paraquat in both lung slices and isolated alveolar epithelial type II cells. Extensive structure–activity relationships have been carried out to determine the key features of molecules necessary to inhibit pulmonary polyamine accumulation [5]. This work was aimed both at characterization of the polyamine uptake system as well as with a view to designing potential antidotes for paraquat toxicity. Some key structural characteristics of the pulmonary polyamine uptake system, which have been revealed from these studies are that in order to be a good inhibitor a molecule requires: (a) one or, preferably, two charged nitrogen-containing cationic groups; (b) a distance equivalent to 4–7 methylene units between the cationic centres, (c) no carboxyl groups adjacent to a cationic centre; (d) steric factors or ionic interactions which may result in hindered access to the cationic-binding site [5]. In more recent studies, we have also shown that disulphides such as cystamine may also be accumulated by this system and subsequently may serve an important role as antioxidants in the lung [6]. The lung is a heterogeneous organ comprising over 40 cell types, but recent studies have suggested that the pulmonary polyamine uptake system resides primarily in alveolar type I and II cells and possibly also in Clara cells [7, 8].

Polyamine uptake systems in tumour cells and their exploitation for cancer chemotherapy

During the course of our studies on the characterization of the pulmonary polyamine uptake system, we noted that the antitumour drug, methylglyoxal-bis(guany1hydrazone) (MGBG) [9], a structural analogue of spermidine, was a potent inhibitor of the pulmonary polyamine uptake system [8]. In further studies, we observed that MGBG was also a substrate for the pulmonary polyamine uptake system [10]. A study of the literature revealed that the uptake of MGBG into various cell types including mouse ascites L1210 tumour cells, HeLa, rabbit reticulocytes, non-mammalian reticulocytes [11], Ehrlich ascites [12], and cultured human lymphocyte leukaemic cells [13], had been documented and characterized to varying extents. It was apparent to us that there were marked similarities between these documented uptake systems for MGBG and the pulmonary polyamine uptake system. Over the last few years, polyamine uptake systems have been further documented in a number of other tumour cells, including rat prostatic tumour cells [14], neuroblastoma cells [15], B16 melanoma cells [16], human prostatic cancer cells (HL60) [17] and human colon and lung tumour cell lines (A. M. Collagher & G. M. Cohen, unpublished work).

An intriguing feature of the polyamine uptake system which merits particular note is that it may be dramatically increased by pretreatment with α-α-difluoromethylornithine (DFMO). DFMO is a specific enzyme-activated irreversible inhibitor of ornithine decarboxylase, the initial and often rate-limiting step in polyamine biosynthesis [18]. This inhibition leads to a marked decrease in intracellular polyamines, as a result of which the cell tries to compensate by stimulating the uptake of extracellular polyamines. Using DFMO pretreatment, it has been shown that cells may concentrate MGBG intracellularly resulting in a greater than 1000-fold intracellular to extracellular concentration [13]. We believe that this marked intracellular accumulation of polyamine-related structures may be exploitable in cancer chemotherapy.

Clearly, structural analogues of the natural polyamines, such as MGBG, can be selectively concentrated in tumour cells. In addition, structural modification of polyamines, such as substitution at the central nitrogen of spermidine with cytotoxic compounds, which in turn do not interfere with the
transport of the polyamines [19] may be concentrated intracellularly in high cytotoxic concentrations in tumour cells possessing the polyamine uptake system. Such chemicals should be selectively toxic to those cells possessing the uptake system. Two other features of these molecules will add to their selectivity. First, the linkage with the polyamines will target the cytotoxic moiety directly to the DNA, so enhancing its localization in close proximity to the key target macromolecule, i.e. DNA [20]. The other key feature relates to the pharmacokinetics and the mode of administration of these agents. Problems have arisen with MGBG owing to unacceptable host toxicity, although it showed extremely good therapeutic responses in early clinical trials [21]. We suggest that this toxicity may be largely due to the administration of the free thiol. The disulphide may then be accumulated in a selective manner in the tumour cells. The patient may have been previously primed with DFMO if this results in a selective depletion of polyamines in the tumour cells compared with critical populations. DFMO infusions should obviate unnecessary toxicity caused by peak concentrations reached in different tissues. Such continuous intravenous infusions have been shown to lower the toxicity of a number of chemicals, including the pulmonary toxicity of bleomycin as well as enhancing its antitumour activity [22].

Potential exploitation of polyamine uptake system in targeting radioprotectors

We have recently found that the radioprotective compound WR2721 [S-2-(3-aminopropylamino)ethyl phosphorothioate], can act as a substrate for the uptake system [23]. However, the separation of the nitrogen atoms in WR2721 by three methylene groups suggested that this molecule would be a relatively poor substrate. The ability of a series of S-2-aminoalkylamino)ethyl phosphorothioates to inhibit competitively the uptake of putrescine into the lung was therefore measured and used as an indirect measure of the ability of the radioprotectors to be accumulated into lung. The compounds used were WR2721, S-2(4-amino-2-butanlamino)ethyl phosphorothioate (S-AHEP) and S-2-(7-aminoheptylamino)ethyl phosphorothioate (S-AHEP) [23]. Their inhibitory potency increased as the number of methylene groups between the nitrogen atoms increased. These compounds inhibited putrescine accumulation competitively, supporting the suggestion that they are accumulated by the same uptake system. WR2721 is rapidly dephosphorylated by alkaline phosphatase to form the free thiol N-2-mercaptopropyl-1,3-diisopropylthiourea [24]. Such dephosphorylated radioprotectors may then be oxidized to the disulphide in the absence of dithiothreitol (DTT) [23], by analogy with cystamine [6]. The disulphide may then be accumulated in a similar manner to that described for the uptake of cystamine. Our results suggested that the transport receptor in the lung recognized the diamine structure in either its free thiol or disulphide form, although chemicals act as more effective ligands in a disulphide form probably as a result of the greater separation between the nitrogens [23].

These results, together with a greater understanding of the structural requirements for transport by this uptake system, suggest it may be possible to target a wide variety of chemicals to the alveolar type I and type II cells, and Clara cells of the lung [7, 8]. It is clear that by altering the number of methylene groups separating the amine groups in the substrate, it is possible to alter the affinity of the chemical for the uptake process. It seems reasonable to argue that S-ABEP and S-AHEP may be more effectively accumulated by the lung than WR2721, thereby providing a more targeted and consequently more effective radioprotection [23]. Again we suggest that to optimize the therapeutic benefit of the polyamine uptake system, the radioprotector should be infused slowly at concentrations approximating the K_m of the uptake system.

The lung is not the only organ that has the ability to accumulate polyamines. Theoretically, it should be possible to target sulphur-containing chemicals to other cell types with the uptake system so as to alter their thiol status and thereby protect these cells from oxidative stress.

Utilization of the uptake system in antiprotozoal therapy

Polyamine metabolism has long been recognized as a potential therapeutic target in trypanosomes [25]. DFMO has been shown to cure mice infected with a virulent strain of Trypanosoma brucei brucei [25]. DFMO has been used in combination with bleomycin, an anticancer agent with a complicated glycopeptide structure with a number of polyamines attached, so it may possibly utilize the polyamine uptake system. This combination has been shown to be synergistic in treating mice infected with Trypanosoma brucei, but the effect can be reversed by co-administration of polyamines [26, 27]. It is also worthy of note that a number of compounds used in antimarial therapy such as primaquine, chloroquine and pentamidine are structurally related to polyamines and have been shown to block the pulmonary polyamine uptake system [28].

In summary, we have described a pulmonary polyamine uptake system which has marked similarities to a polyamine uptake system present in a number of different cell types, in particular, certain tumour cells. We have described how this uptake system may be exploited to design novel cytotoxic cancer chemotherapeutic agents, as well as designing more efficacious radioprotective agents.

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Bioprecursor approach to drug targeting

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The ability to direct a pharmacological agent to its biological target in order to reduce its side-effects while increasing its efficacy has been a medicinal chemist's dream for a long time (and still is). Many different approaches have concentrated on designing complex delivery systems such as mammalian cells, liposomes, liposomas incorporating antibodies, and synthetic polymers [nanoparticles] [1]. The function of these delivery systems is to neutralize and protect the native drug in the systemic circulation and eventually to guide it towards the target organ.

This presentation deals with chemical approaches in which the pharmacological agent is circulating and distributing freely through the whole organism in an inactive form. Through the action of an enzyme (see below) this inactive compound will be transformed into the active drug in the systemic circulation and eventually to guide it towards the target organ. This presentation deals with chemical approaches in which the pharmacological agent is circulating and distributing freely through the whole organism in an inactive form. Through the action of an enzyme (see below) this inactive compound will be transformed into the active drug in the systemic circulation and eventually to guide it towards the target organ. This presentation deals with chemical approaches in which the pharmacological agent is circulating and distributing freely through the whole organism in an inactive form. Through the action of an enzyme (see below) this inactive compound will be transformed into the active drug in the systemic circulation and eventually to guide it towards the target organ.

A brain-specific carrier for dopamine

Dopamine itself does not cross the blood-brain barrier. Its synthesis, which is deficient in Parkinson's disease, is stimulated by the administration of L-dopa. Although this is a generally well accepted and efficient treatment, direct delivery of dopamine to the brain in a sustained and controllable way would represent a valid and attractive alternative. Bodor & Simpkins [2] described an amine-carrier system based on a redox reaction of a dihydroxyproline to pyridinium salts. In short, the amine (for instance phenethylamine) is coupled via an amide link to N-methyl,1,4-dihydroxyproline,3-carboxylate. When administered to an animal by a systemic route this lipophilic conjugate crosses the blood-brain barrier. Oxidation of the dihydroxyproline to the pyridinium salt in the central nervous system locks the new conjugate in the brain; chemical or enzyme-catalyzed hydrolysis of the slightly more reactive amide bond will then slowly liberate the amine. When applied to dopamine, with the appropriate adjustments, no increase of dopamine concentration in the brain of animals given the conjugate could be demonstrated. However, there was a marked drop of serum prolactin levels, which is consistent with a stimulation of dopamine receptors in the anterior pituitary gland.

Dopamine renal-specific pro-drug

Dopamine exerts a vasodilatory function by binding to a specific receptor in the kidney. Such an action is beneficial in arterial hypertension as demonstrated by slow intravenous infusion of dopamine. However, the therapeutic window is quite narrow and at higher infusion rates, dopamine activates α-adrenergic receptors resulting in a pressor effect. A dopamine derivative which accumulates specifically and liberates dopamine in the kidney would overcome this problem. The kidney has an active uptake system and metabolism of γ-glutamyl derivatives of amines, amino acids and peptides. This is due to the presence of a high activity of γ-glutamyl transpeptidase activity in the brush-border cells of the proximal tubules. Wilk et al. [3] synthesized γ-glutamyl-dopa and studied its biological and pharmacological effects. They demonstrated that γ-glutamyl-dopa accumulates in the kidney, liberates dopa and, through the action of aromatic amino acid decarboxylase (AADC), generates dopamine locally. For instance, after a dose of 0.5 μmol/kg in rats, the kidney concentration of dopamine was six times higher in animals given the γ-glutamyl conjugate than in animals given dopa, and the elevation was of longer duration, in agreement with a slow release as dopamine is metabolized very rapidly. This increase of dopamine is accompanied by a marked increase of renal plasma flow.

Dual-enzyme activated inhibitors of dopamine synthesis and metabolism

If α-methyl-dopa had been designed with the mechanism of action as understood today in mind, it would be a perfect example of this class of compounds. Instead of being a competitive inhibitor of AADC, as originally believed, α-methyl-dopa is transformed into α-methyl-noradrenaline, an α-agonist, by the combined action of AADC and dopamine-β-hydroxylase. Two examples of designed bioprecursors of enzyme-activated inhibitors have been described recently.

1. Bioprecursors of AADC inhibitors [4]. α-Mono-fluoromethyl-dopa is a very powerful, time-dependent, irreversible inhibitor of AADC. It blocks the biosynthesis of all

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