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Hydrolytic Enzymes Targeting to Prodrug/Drug Metabolism for Translational Application in Cancer

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Review Article

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Abstract

A variety of approaches are under development to improve the effectiveness and specificity of enzyme activation for drug metabolism on tumour cell targeting for cancer treatment. Many such methods involve conjugates like monoclonal antibodies, substrate specificity and offer attractive means of directing to tumour toxic agents such as drugs, radioisotopes, protein cytotoxins, cytokines, effector cells of the immune system, gene therapy, stem cell therapy and enzyme therapy for therapeutic use. Hydrolytic enzymes belong to class III of enzyme classification and play an important role in the drug metabolism towards the treatment of cancer. Hydrolases helps drugs for metabolic efficiency to target on cancer cell since it is involved in the hydrolytic reaction of various biomolecules and compounds. The prodrug is designed to be a substrate for the chosen enzyme activity. A number of prodrugs have been developed that can be transformed into an active anticancerous drugs by enzymes of both mammalian and non mammalian origin. The basic molecular biochemistry, biotechnological processes and other information related to enzyme catalysis, has a major impact for the production of efficient drugs. In the current review some of the Hydrolases has been discussed which play a significant role towards prodrug to drug metabolism for cancer treatment.

Keywords: Carboxylesterases; Alkaline phosphatases; β-Glucosidase; β-glucuronidase; Carboxy peptidase; Matrix metalloproteinases; Epoxide hydrolases; Cancer; Hydrolases; Drug metabolism

Abbreviations: ADEPT: Antibody-Directed Enzyme Prodrug Therapy; CEs: Carboxylesterases; rCE: rabbit CE; ALP: Alkaline Phosphatases; ACE: Angiotensin-I Converting Enzyme; C-terminal: Carboxy-Terminal; MMPs: Matrix Metalloproteinases; EHs: Epoxide Hydrolases.

Introduction

The structural and functional unit of life the cell when it has been transformed it is dangerous to human life. The cancer which is formed from uncontrolled growth of cell and dysfunction of various macromolecules including genes and proteins. Enzymes are the proteins which are not only enhance the rate of reactions but also involved in drug metabolism for three phases trials of drug testing. A variety of approaches are under development to improve the effectiveness and specificity of enzyme activation for drug metabolism (usually Phase I trials) on tumour cell targeting for cancer treatment. Many such methods involve conjugates like monoclonal antibodies, substrate specificity and offer attractive means of directing to

tumour toxic agents such as drugs, radioisotopes, protein cytotoxins, cytokines, effector cells of the immune system, gene therapy, stem cell therapy and enzyme therapy for therapeutic use.

Hydrolytic enzymes belong to class III of enzyme classification and play an important role in the drug metabolism towards the treatment of cancer. Hydrolases helps drugs for metabolic efficiency to target on cancer cell. The prodrug is designed to be a substrate for the chosen enzyme. A number prodrugs have been developed that can be transformed into active anticancerous drugs by enzymes of both mammalian and non mammalian origin. In the current review some of the Hydrolases has been discussed which play significant role towards prodrug to drug metabolism.

Carboxylesterases (CEs)

Carboxylesterases are belonging to EC 3 1 1 1 of Enzyme classification and are ubiquitous enzymes responsible for the detoxification of ester-containing xenobiotics in which hydrolysis reaction results in the formation of the corresponding carboxylic acid and alcohol. CEs are able to cleave ester linkages in many clinically useful drugs, such as heroin, cocaine, mepridine, licodaine, etc [1]. CEs are essentially involved in drug metabolism and also in drug activation and other biological processes [2]. CEs are also involved in prodrug activation as they can hydrolyze prodrugs such as lovastatin to active metabolites [1,2].

For an example CPT-11 {Irinotecan; 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin} is a prodrug that is activated by CEs to generate SN-38 (7-ethyl-10-hydroxycamptothecin), a potent topoisomerase I poison [3]. CPT-11 has demonstrated remarkable antitumor activity in human tumor xenograft models and Undergone trials for both Phase II and Phase III clinical trials in adults and children [3-6]. Irinotecan (CPT-11) is an anticancer alkaloid registered for the treatment of metastatic colorectal adenocarcinoma [7]. Mice may represent a more accurate model for antitumor studies with this drug Irinotecan and other agents metabolized by CEs. According to pharmacokinetic data, four to fivefold more CPT-11 was required to induce regressions in human Rh30 xenografts grown in esterase-deficient scid mice, as opposed to those grown in scid animals. It is been reported that activation and antitumor activity of CPT-11 in plasma esterase-deficient mice [8]. CEs are almost identical, human CE converts CPT-11 100–1000-fold less efficiently [9]. In Rh30 human rhabdomyosarcoma cells, expression of the rabbit enzyme was associated with more rapid tumor regression and a better prevention of tumor recurrence *in vitro* [9,10].

After administration of a prodrug, antitumor activity occurs since high levels of active metabolite will be produced within the milieu of the tumor. This methodology has been applied to activate CPT-11 using hiCE (human intestinal CE) and rCE (rabbit CE) against a melanoma tumor model [11,12]. Enzyme prodrug therapy involves targeting of an antibody–enzyme fusion protein to tumor cells. In this antibody-directed enzyme prodrug therapy (ADEPT) approach, an antigen that is specifically expressed on tumor cells is used to selectively deliver an enzyme that can activate a prodrug [12].

Alkaline Phosphatase (ALP)

Alkaline phosphatases belongs to EC 3 1 3 1 of enzyme classification. ALP catalyzes the removal of phosphate groups from a diverse class of compounds, including nucleotides, proteins and alkaloids. ALP is ubiquitously expressed in many tissues with the highest expression in the liver. [13]. the differential expression of various ALP isozymes in liver and bone has been used as a diagnostic marker for various cancers and renal dysfunction.

Many established chemotherapeutic drugs have been phosphorylated to form much less active prodrugs suitable for regeneration by the enzyme alkaline phosphatase, which is hydrolytically, removes phosphates. The phosphorylated version of the first drug to be used, etoposide, is more than 100-fold less toxic to a human carcinoma cell line [14]. ALP from calf intestine used to convert etoposide phosphate into clinically approved anticancer drugs etoposide. The reason is that the phosphorylated drug derivatives were much less cytotoxic to the H291 human lung adenocarcinoma cell line since phosphorylated drug cannot penetrate cell membranes. When alkaline phosphatase was directed to tumour cells in vitro and in vivo by the tumour-specific monoclonal antibody L6, significant immunospecific antitumour activity was seen. The cytotoxic activities of the prodrugs activated by 100 fold on cells which were treated with binding conjugate L6 ALP before exposure. This suggests that L6ALP able to convert the prodrugs cytotoxic anticancer drugs.

Another approach for prodrug activation by using bispecific antibodies successfully to deliver cytotoxins such as saporin to tumour cells [15]. This approach has also been incorporated in a prodrug activation system to deliver alkaline phosphatase to a Hodgkin's disease-derived tumour cell line [16]. An anti-alkaline

phosphatase/anti CD30 bispecific antibody, expressed from a hybrid hybridoma, was preincubated with the enzyme before the whole assembly was tested on antigen-positive cells.

Various ALP isozymes in liver and bone have been used as a diagnostic marker for various cancers and renal dysfunction [17]. ALP is believed to be responsible for the activation of several clinically used phosphate prodrugs including fosfluconazole, fosphenytoin, fosprepitant, amifostine, clindamycinphosphate, estramustine phosphate, and etoposide phosphate. Most of these prodrugs were developed to overcome the solubility problems of parent drugs and are for parental administration while some are for the targeted treatment of cancer. Amifostine (WR-2721, S-2 [3-aminopropylamino]-ethylphosphorothioic acid) was developed as a protective agent against radiation- and chemotherapy- induced cellular injury [18]. The membrane-bound alkaline phosphatase that activates amifostine is highly active in the cell membrane of normal endothelial cells but not in the cell membranes and neovascular capillaries of tumors, enabling selective protection of nontumor cells [19,20].

ALP-mediated prodrug activation has been applied to cancer imaging and therapy. The quinazolinone molecule precipitates and is concentrated and permanently trapped within the extracellular spaces of targeted solid tumors. The prolonged residence time of the quinazolinone molecule should permit the noninvasive detection and therapy of tumors. The prodrug is hydrolyzed to a much less water-soluble, radiolabeled quinazolinone ^{125}IQ2-OH by ALP, which is overexpressed on the exterior surfaces of tumor cell plasmamembranes [21]. *S*-2-(3-Aminopropylamino)-ethylphosphorothioic acid (amifostine, WR-2721) is a clinically used prodrug that is dephosphorylated by membrane-bound alkaline phosphatases to the chemoprotective thiol WR- 1065 [22].

β -Glucosidase

β-Glucosidase (**EC no 3 2 1 21)** hydrolyses β 1-4 linkages between two glucoses or glucose-substituted molecules (such as cellobiose) [23]. β-Glucosidase also plays an important role in the treatment of Gaucher's disease (resulting from a deficiency of β-glucosidase) in which accumulation of glycoceramides takes place in the lysosomal tissues [24]. β-Glucocerebrosidase is also called acid β-glucosidase (EC 3.2.1.45) 497 amino acids in length and has a molecular weight of 59700 Daltons. Targeting mammalian enzymes for metabolism of drugs, which are usually noncytotoxic and non-immunogenic, internally to a particular cell compartment where they can catalyse a reaction resulting in cell death [25].

In the approach antibody-guided enzyme nitrile therapy system, known as AGENT, is based upon the enzyme β-glucosidase conjugated to a tumour-specific monoclonal antibody. Human tumours do not express a β-glucosidase which is capable of activating amygdalin, so with the targeted system, cyanide can diffuse into tumour and surrounding cells and kill them by inhibiting mitochondrial respiration. This work gave significant results of cell-bound antibody- β-glucosidase conjugate can enhance the cytotoxicity of amygdalin to that of the level of cyanide alone in vitro. This results in a 1,000-fold enhancement of toxicity [25].

β-Glucuronidase

Human β-glucuronidase (EC3.2.1.31) is a lysosomal enzyme that plays an important role in the degradation of glucuronic acid-containing glycosaminoglycans, such as heparin sulfate, chondroitin sulfate and dermatan sulfate [26].

An endogenous extracellular β-glucuronidase is present at high levels in necrotic tumors, where glucuronide prodrugs could potentially be used as a monotherapy. Moreover, in the case of non-necrotic areas of tumors where the enzyme concentration is low, exogenous β-glucuronidases can be delivered to the same tumor tissues using antibody-directed enzyme prodrug therapy (ADEPT) and GDEPT strategies [27].

Cancer tissues have been targeted by a wide variety of glucuronide prodrugs exploiting the presence of high concentrations of β-glucuronidase as the enzyme is not present in the general circulation. Upon β-glucuronidase-catalyzed hydrolysis, the 4-hydroxy benzyl carbamate-doxorubicin intermediate undergoes self-immolative 1, 6-elimination to release the active doxorubicin drug and more effective prodrug activation can be achieved in vitro using liposomes. In this case large aggregates consisting of a liposome with some 400 Fab' fragments and 20 β-glucoronidases has been made [28].

β-Glucuronidase prodrugs have been developed to improve the bioavailability of antitumor agents. Most prodrugs consist of the general structure drug-spacer-glucuronic acid. Upon prodrug hydrolysis by β-glucuronidase, fragmentation of the self-immolative spacer occurs and the drug is released. The tetra-*n*-butyl ammonium salt of (*p*-di-2-chloroethylaminophenyl- β-D-

glucopyranoside) uronic acid (BHAMG) is a nitrogen-mustard prodrug that is activated by β-glucuronidase to N, N-di-(2-chloroethyl)-p-hydroxyaniline mustard [29]. Beta-glucuronidase has already been proven to be useful in tumour specific bioactivation of glucuronide prodrugs of anticancer agents [30].

Many prodrugs have been used successfully in the clinic; examples include oseltamivir in anti-influenza therapy, enalapril in anti-hypertension therapy, capecitabine in cancer therapy, and omeprazole in the treatment of peptic ulcer. A key step in prodrug design is the incorporation of an activation mechanism that can convert the prodrug into the active species in an efficient and/or controlled manner to meet the needs of a given medical applications.

Temocapril [31] along acting Angiotensin-I converting enzyme (ACE) inhibitor, is quickly metabolized to the active carboxylic acid; dipivefrin [32] available as an ophthalmic solution for the treatment of glaucoma, is hydrolyzed to epinephrine on penetrating cornea [33].

Carboxypeptidase

Carboxypeptidase (EC number 3.4.16 - 3.4.18) is a protease enzyme that hydrolyzes (cleaves) a peptide bond at the carboxy-terminal (C-terminal) end of a protein or peptide.

Carboxypeptidases catalyze the hydrolysis of peptides, resulting in the formation of a shortened peptide and an amino acid [13]. The two substrates of this enzyme are penicillin and H_2O, whereas its two products are carboxylate and 6-aminopenicillanate.

Carboxypeptidases hydrolyze Peptidyl methotrexate derivatives. Methotrexate MTX4 an anticancer drug with broad spectrum activity against L1210 leukemia cells invitro. The alanyl MTX less active than MTX against cell line. Both carboxypeptidase A and B are affected the hydrolysis of MTX-ala resulting in stable increases cytotoxic activities [34].

A MAb–CPG2 conjugate has been used for prodrug activation. CPG2 was able to effect the hydrolysis of the nitrogen mustard 1 to form benzoic acid mustard 2, which is against the JAR human choriocarcinoma cell line (IC_{50} 20M) [35].

Matrix Metalloproteinases

Matrix metalloproteinases (MMPs), also known as matrixins, are calcium-dependent zinc-containing endopeptidases [36]. MMPs belong to EC 3, 4 subtypes groups mentioned in Table 1 [37].

Enzyme	Alternative Name	EC Number	Substrates
MMP-1	Interstitial Collagenase	3.4.24.7	Collagens(**I, II, III, VII, VIII,** and **X**); gelatin; aggrecan; L-Section; **IL-I** beta; protteoglycans; entactin; ovostatin; **MMP-2; MMP-9**
MMP-2	Gelatinase **A**	3.4.24.24	Collagens(**IV, V, VII,** and **X**); gelatin
MMP-3	Stromelysin-1, Proteoglycanase	3.4.24.17	Collagens(I, II, III, VII, VIII, and X); gelatin; aggrecan; perlecan; decorin; laminin; elastin; caesin; osteonectin; ovostatin; entactin; plasminogen; **MBP; IL-** beta; **MMP-2/TIMP-2; MMP-7; MMP-8; MMP-9; MMP-13**
MMP-8	Neutrophil collagenase	3.4.24.34	Collagens(**I, II, III, V, VII, VIII, and X**); gelatin; aggrecan; fibronectin
MMP-9	Gelatinase **B**	3.4.24.35	Collagens(**IV, V, VII, X, and XIV**); gelatin; entactin; plasminogen; aggrecan; elastin; fibronectin; osteonectin; **MMP; IL- I** beta
MMP-13	Collagenase-3	_	Collagens(**I, II, III, IV, IX, X and XIV**); gelatin; plasminogen; aggrecan; perlecan; fibronectin; osteonectin; **MMP-9**
MMP-14	**MTI-MMP**	3.4.24.80	Collagens**(I, III);** gelatin; casein; fibronectin; Laminin; vitronectin; entactin; proteoglycans; **MMP-2; MMP-13**
MMP-18	Xenop-us Collagenase-4	_	Not Known
MMP-22	Chicken **MMP(C-MMP)**	_	Not Known

Table 1: Matrix Metalloproteinases and their substrates.

They play an important role in the degradation of extracellular matrix components, such as collagen, laminin, fibronectin, and elastin. MMPs also cleave a variety of proteins, such as growth factor receptors and cell adhesion molecules, which may be an important for tumor growth and survival [38,39]. Because of their significant role in tumor angiogenesis, invasion, and metastasis, MMPs have been target edinpotential therapeutic strategies for a number of disease conditions including cancer, arthritis, glomerulonephritis, periodontal disease and ulcers [40]. Delivery of doxorubicin to tumor tissues was investigated using its peptide conjugates as prodrugs activated by the combined proteolytic action of MMP-2, MMP-9, and MMP-14. Doxorubicin is a potent anthracycline used for the treatment of various types of cancer. It causes cytotoxicity through topoisomerase II-mediated DNA breaks [41].

Epoxide Hydrolases

The epoxide hydrolases (EHs) belongs to hydrolases family of EC 3.3.2.3, which hydrate simple epoxides to vicinal diols, and they hydrate arene oxides to trans-dihydrodiols. The major role of the microsomal enzyme is the inactivation of xenobiotic compounds by *trans*-addition of water to epoxides [42]. Structure and catalytic activities of these enzymes have reviewed by Fretland, *et al*. Several anticancer agents, including the antiangiogenic drug TNP-470 and bropirimine are metabolized to reactive epoxides or arene oxides by CYP450 and detoxified by the microsomal epoxide hydrolase [43].

Prodrug activation can be achieved through enzyme-mediated hydrolytic or oxido-reductive processes (including transferases, hydrolases, and lyases) while activation of some prodrugs may proceed through pure chemical nonenzymatic processes.

Therefore it is necessary to enhance the activity of Hydrolases for their efficient drug metabolism. For an example our recent findings with our current experiments of Brain tumor cell lines mutant p53 LN 229 and U251 have shown lower CE activity but they showed significant higher CE activity with $LiCl_2$ treatment (not reported). This exactly confirms that Lithium is positive modulator which enhances CE activity and efficient anticancerous drug metabolism. Therefore it can be conjugated with anticancerous drugs for their metabolic efficiency and targeting on Brain tumor cells for better treatment. Thus, the studies on the carboxyl esterases enzyme's activities of brain tumors could be useful for diagnostic as well as to predict the prognosis of the cancer and their CE activities to improve drug metabolism and therapeutic purposes.

It is necessary for several prodrug-activating enzymes the organ/tissue/organelle distribution in humans and levels between normal and tumor tissues has to be further investigated. In addition to this, more work needs to be performed with a series of prodrug analogs to determine optimal substrates in terms of high specific activity and fast enzyme turnover rates for prodrug-activating enzymes resulting in fast cleavage of the prodrug inside the tumor cells [44].

Therefore the current study provides the chance of solving the tumor problem and in future these properties has advances molecular biochemistry and modern methods to understand pathophysiology of the tumor types with their enzyme activity for prodrug/drug metabolism in translational research and applications.

This information confirms that efficiency of the drug activation will be high with conjugates and activity of enzymes is more than systemic administration of drug itself and Enzyme-mediated hydrolytic activation of prodrugs become effective tools against various diseases for better treatment [37,45].

The basic molecular biochemistry, biotechnological processes, molecular modeling by bioinformatics and other information related to genetic /protein / metabolic engineering, enzyme catalysis, has a major impact on the pharmaceutical industries for production of efficient drugs.

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