

COMPETITIVE INHIBITORS OF ENZYMES AND THEIR THERAPEUTIC APPLICATION

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Summary. *Enzymes catalyze virtually every biochemical process in the cell. The usefulness of the most important pharmaceutical agents, antimetabolites, is based on the concept of competitive enzyme inhibition. The antimetabolites are structural analogues of normal biochemical compounds. As competitive inhibitors they compete with the naturally substrate for the active site of enzyme and block the formation of undesirable metabolic products in the body. Antibacterial, antiviral and anticancer pharmaceutical agents are among numerous examples of antimetabolites. Sulfa drugs, sulfanilamide, structural analogs of amino acids (cycloserine, L-fluoroalanine), folic acid antagonists (4-amino-10-methyl folic acid=methotrexate), analogues of purine and pyrimidine (6-mercaptopurine, allopurinol, 5-fluorouracil, 5-azacytidine), inhibitors of polyamine biosynthesis (difluoromethyl ornithine, methylglyoxal-bis (guanyl hydrazone)) are the most used in modern chemotherapy. The use of enzyme inhibitors, antimetabolites, beside the therapeutic significance has also provided valuable informations about enzyme mechanisms and has helped to define some metabolic pathways.*

Key words: *Enzyme inhibitors, sulfa drugs, antimetabolites, folic acid antagonists, purine and pyrimidine analogues, polyamines antagonists, chemotherapy*

Introduction

Enzymes are the reaction catalysts of biological systems which accelerate and direct specific biochemical reactions. Great specificity (speciality) of enzymes is a very important biological phenomenon which assures high coordination to yield a harmonious interplay among many different metabolic activities necessary to sustain life.

Modification of enzyme activity

It is well known that activities of intracellular and extracellular enzymes depend on numerous constituents of medium or circumstances. The most important factors which influence enzyme activity are presented by enzyme concentration, the amount of specific enzyme substrate, electrochemical reaction of medium for enzyme activity (pH), the presence of activators (specific or nonspecific) as well as the presence of inhibitors (naturally occurring or intended for specific purpose, commonly used as chemotherapeutic agents).

Inhibitors of enzymes

The inhibitors of enzyme activity are chemical substances, which in small quantity decrease the activity of enzymes in a specific chemical way. As a result of the

inhibitor - enzyme interaction enzyme-inhibitor a complex is formed: once bound, the enzyme cannot convert the inhibitor to products. The existence of specific naturally occurring enzyme inhibitors, like antithrombin, antipepsin and antitrypsin, controls the enzyme activity in human the body and under physiological circumstances assures their intracellular and extracellular action. Among the naturally occurring enzyme inhibitors there are also intermediary products formed during some metabolic pathways. Product inhibition provides a limited mean of control or modulation of substrate flux through the pathway. If one or more enzymes are allosteric enzymes particularly sensitive to product inhibition, the output of end product of the pathway will be suppressed (1).

Mechanism of competitive inhibitors action

Competitive inhibitors are compounds that resemble structurally the substrate and compete with substrate for the active site of an enzyme to form an enzyme-inhibitor complex. Once the inhibitor occupies the active site of enzyme it prevents binding of substrate and abolishes the formation of normal metabolic product (1,2). Inhibitor binds reversibly the enzyme and because of that the competition can be decreased simply by adding more substrate. When enough substrate is present the

probability that an inhibitor molecule will be bound is minimized, and enzyme reaction exhibits a normal $V_{\max}$. In the presence of competitive inhibitor Michaelis-Menten constant, K_m will increase (2).

Application of competitive inhibitors in medicine

Enzymes catalyze virtually every process in the cell and it should not be surprising that enzyme inhibitors are among the most important pharmaceutical agents known. Classic example of competitive inhibitor of *succinate dehydrogenase* is malonic acid / $\text{HOOC}-\text{CH}_2-\text{COOH}$ /, structural analog of succinic acid/ $\text{HOOC}-\text{CH}_2-\text{CH}_2-\text{COOH}$ / (Fig. 1). In the presence of malonic acid succinate dehydrogenase activity, one of citric acid cycle enzymes, is inhibited, and the reaction of citric cycle is blocked, respectively (1-2).



Fig. 1.

Most modern drug therapy is based on the concept of enzyme inhibition

Competitive inhibition is used therapeutically to treat patients who have ingested methanol. In the human body ingested methanol is converted into formaldehyde by the action of the enzyme alcohol dehydrogenase. Formaldehyde damages many tissues, and blindness is a common result because the eyes are particularly sensitive. The therapy of methanol poisoning is intravenous infusion of ethanol; ethanol competes effectively with methanol as a substrate for alcohol dehydrogenase. Ethanol is also substrate for alcohol dehydrogenase forming acetaldehyde and acetate. Intravenous infusion of ethanol slows down the formation of formaldehyde so that most of methanol can be excreted harmlessly by urine.

The application of therapeutic drugs as a specific enzyme inhibitors, inhibits the playing of unwanted metabolic pathways in the body and for that reason these drugs are named **antimetabolites** (2). Antibacterial, antiviral and antitumor drugs belong in the group of these drugs. The administration of those drugs to the patients causes limited toxicity because there are few critical metabolic pathways that are unique to tumors, viruses, or bacteria; hence drugs that kill these organisms will often kill host cell. Antimetabolites are compounds with some structural difference from the natural substrate and belong in the group of competitive enzyme inhibitors. Sulfa drugs, structural analogs of amino acids, folic acid antagonist, analogs of purines and pyrimidines belong to this group of enzyme inhibitors.

Application of sulfa drugs in medicine

Modern chemotherapy had its beginning in compounds with the general formula $\text{R}-\text{SO}_2-\text{NHR}'$. The simplest member of sulfa drugs is sulfanilamide, an antibacterial agent. Sulfanilamide is an antibiotic useful in the treatment of some kidney infection. As a structural analog of p-aminobenzoic acid (PABA) (Fig. 2), sulfanilamide inhibits bacterial growth. PABA is a structural part of folic acid, which is composed of pteridine, p-aminobenzoic acid and glutamic acid. Some kinds of bacteria require folic acid for their growth and division. As the structural analog of p-aminobenzoic acid sulfanilamide is a competitive inhibitor for bacterial dihydrofolate synthetase. Thus bacteria are starved of the required folate and cannot grow and divide. Sulfanilamide is antibiotic useful in the treatment of some kidney infection. This drug is highly toxic to bacteria that must synthesize their own folic acid. Since humans require folate from dietary source, the sulfanilamide is not harmful at the doses that kill bacteria (2).

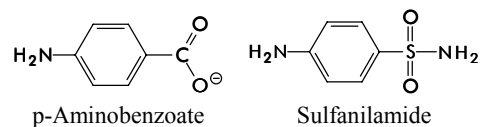
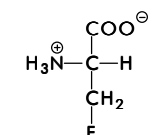


Fig. 2.

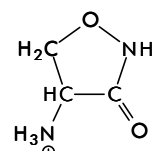
Structural analogs of amino acids

Structural analogs of amino acids are used as antibacterial drugs. D-amino acids, like D-alanine and D-glutamic acid, occur as structural part of bacterial cell walls and peptide antibiotics. D-Amino acids arise directly from the L isomers by the action of amino acid racemases, which have pyridoxal phosphate as a required cofactor. Racemisation of amino acids is uniquely important to bacterial metabolism, and enzyme such as alanine racemase represent prime targets for pharmaceutical agents. One such agent, L-fluoroalanine (Fig. 3), is being tested as an antibacterial drug. Cycloserine, analog of serine (Fig. 4), is already used to treat urinary tract infection and tuberculosis. In modern psychiatry cycloserine is frequently used as a therapeutic agent (3-5). As a structural analog of serine, cycloserine inhibits the synthesis of sphingosine, sphingomyelin respectively (6).



L-Fluoroalanine

Fig. 3.



Cycloserine

Fig. 4.

Folic acid antagonists-antifolates

Folic acid, folacin or pteroyl glutamic acid belong to the group of water soluble vitamins. Fresh leafy green

vegetables, cauliflower, kidney and liver are rich sources of folic acid. The physiological function of folic acid coenzymes is in the synthesis of purine nucleotides and thymine, precursors in the synthesis of RNA and DNA intracellularly, respectively. The folic acid coenzymes are specifically concerned with biochemical reactions involving the transfer and utilization of the single carbon (C_1) moiety. Before functioning as a C_1 carrier, folic acid must be reduced, first to 7,8-dihydrofolic acid (H_2 - folate) and then to the tetrahydro compound (H_4 - 5,6,7,8-tetrahydrofolic acid) catalyzed by folic acid reductase which uses NADPH as hydrogen donor. The participation of folic acid coenzymes in reaction leading to synthesis of purines and to thymine, the methylated pyrimidine of DNA, emphasizes the fundamental role of folic acid in the growth and replication of cells. Cancer cells grow more rapidly than the cells of most normal tissues and thus they have greater requirements for nucleotides as precursors of DNA and RNA synthesis. Consequently, cancer cells are generally more sensitive to inhibitors of nucleotide biosynthesis than are normal cells.

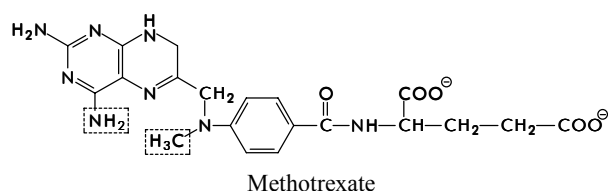


Fig. 5.

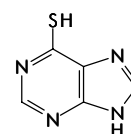
The folic acid antagonists, methotrexate and aminopterin, close structural analogs of folic acid, as anti-tumor agents have found clinical application in the treatment of malignant diseases, especially in the treatment of leukemia in childhood (7-10). Antifolates, folate analogs, aminopterin (4-amino folic acid) and methotrexate (amethopterin, 4-amino-10-methylfolic acid) (Fig. 5) are extremely potent competitive inhibitors of the **dihydrofolate reductase** and **thymidylate synthetase** and because of that inhibits the synthesis of RNA and DNA. Dihydrofolate reductase enzyme is needed for the reduction of dihydrofolic acid (DHF) to tetrahydrofolic acid (THF). Dihydrofolate reductase binds methotrexate about 100 times better than dihydrofolate. Thymidylate synthetase uses methyl-tetrahydrofolic acid as a substrate and transfer methyl group to uracil present in the deoxyuridinemonophosphate (dUMP); in this transmethylation reaction deoxythymidinemonophosphate (dTMP), precursor in the biosynthetic pathway of DNA, is formed which represents the key step in the cell replication and division. Enzyme dihydrofolate reductase binds methotrexate about 100 times better than dihydrofolate. The development of drug resistance to methotrexate appears if the chemotherapy prolongs. Tumor cells that acquired MTX resistances have been found to have an increased number of DNA gene copies encoding for enzyme dihydrofolate reductase. This form of multiple gene reduplication is called gene amplification. The amplified DHFR genes in MTX-resistance cells produce a markedly increased number of copies

of DHFR enzyme, for exceeding the amount of MTX that can be delivered to cell, and thereby allows tumor cell DNA synthesis and tumor regrowth occurs (11).

The application of methotrexate disturbs the metabolism of polyamines in rapidly growing tissues. Inhibition of polyamine oxidase, the key enzyme in biodegradation pathway of spermine and spermidine, induced by methotrexate in regenerating rat liver tissue (12) is probably the consequence of the inhibition of nucleic acids and protein synthesis.

Structural analogs of purine and pyrimidine

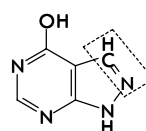
6-mercaptapurine (6-MP), the analog of hypoxanthine and adenine (Fig. 6), is a useful antitumor drug in humans. In the C_6 position of purine ring of hypoxanthine or adenine instead of NH_2 or OH group 6-MP has SH group (1,2). **6-thioguanine (6-TG)** is also thio-purine, analog of guanine. Both thioguanines, 6-MP and 6-TG are converted to nucleotide form by hypoxanthine-guanine phosphoribosyl transferase (HGPRT); their metabolites inhibit a number of enzymes in the purine pathway; some metabolites of thioguanine are incorporated into both DNA and RNA (16, 18). By incorporation and inhibition of nucleic acid synthesis this thioguanine is particularly used in hemotherapy of malignant diseases (10, 13-16). Well known competitive inhibitor of enzyme, as a therapeutic agent in medicine is allopurinol, administered to patients who suffer of gout. Gout is a disease of joints, usually in males, caused by an elevated concentration of uric acid in blood and tissues. The precise cause of gout is not known, but it is suspected to be due to genetic deficiency of one or another enzyme concerned in purine metabolism. The principal enzyme in this metabolism is xanthine oxidase (2).



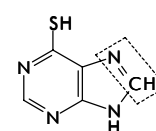
6-Mercaptopurine

Fig. 6.

Allopurinol is structural analog of hypoxanthine (Fig. 7) and represents a competitive inhibitor of xanthine oxidase. When xanthine oxidase is inhibited the conversion of purines into uric acid is stopped; in this case the excreted products of purine metabolism are xanthine and hypoxanthine, which are more soluble in water than uric acid and less likely to form crystalline deposits (4).



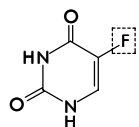
Hypoxanthine (enol form)



Allopurinol

Fig. 7.

5-fluorouracil (5-FU) is a thymine analog in which the ring bound methyl group is substituted by fluorine (Fig. 8). The deoxynucleotide of this compound is an inhibitor of thymidylate synthetase. 5-FU undergoes biotransformation to ribosyl and deoxyribosil nucleotide metabolites. 5-fluorouridine triphosphate is incorporated in RNA and interferes with RNA processing and function.

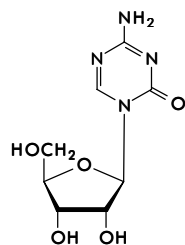


Fluorouracil

Fig. 8.

Incorporation of 5-fluorouracil into deoxyribonucleotide, 5-fluorodeoxyuridine monophosphate, results in irreversible inhibition of enzyme thymidylate synthetase and impossibility of thymidylate (TMP) synthesis. Inhibition of thymidine monophosphate formation blocks DNA synthesis and cell multiplication (10, 16-18). 5-FU is an important anticancer agent in the treatment of different solid tumors.

Cytosine arabinoside, ara-C, also belongs to the group of pyrimidine antagonists. This nucleoside is a specific agent of cell division S-phase especially used in acute nonlymphocyte leukemia therapy and less in the treatment of other malignant hematologic diseases. Intracellularly cytosine arabinoside is metabolised into active form, ara-CTP, which competitively inhibits DNA polymerase, thus blocking DNA synthesis (10).



5-Azacytidine

Fig. 9.

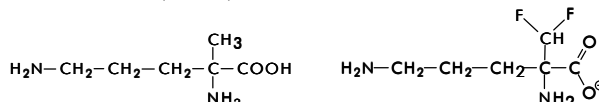
Another structure analog of cytidine is 5-azacytidine (Fig. 9). Intracellularly 5-Aza-C is metabolised into 5-aza-CTP after what it is involved in DNA and RNA synthesis, damaging protein synthesis.

The possibility of application of purine and pyrimidine structural analogs as competitive inhibitors in resembling nucleotide biosynthesis is not limited only to cancer treatment. All rapidly growing cells (including bacteria and protozoa) are potentially sensitive to these agents (1).

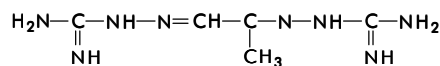
Structural analogs of polyamines as anticancer agents

Polyamines, spermine, spermidine and putrescine are normal cell constituents. Accelerated biosynthesis and accumulation of polyamines are directly connected

to cell growth and proliferation (20,21). The key enzymes in their biosynthesis are ornithine decarboxylase (ODC), which produce putrescine, and S-adenosyl-methionine decarboxylase which is involved in spermidine and spermine synthesis (22). Structural analogs of ornithine α -methyl ornithine (Fig. 10) and difluoromethyl ornithine (Fig. 11) are the most used competitive inhibitors (23-25).

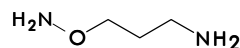
Fig. 10. α -Methylornithine Fig. 11. Difluoro-methylornithine

Methyl-glyoxal-bis (guanyl hidrazone), MGBG, inhibits activity of S-adenosylmethionine decarboxylase (SAMDC), the key enzyme in spermidine and spermine synthesis (26-28). The application of MGBG (Fig. 12) as antiproliferative agent, is used in chemotherapy of malignant diseases; it is based on the fact that accelerated polyamine biosynthesis precedes the accelerated nucleic acid synthesis which provides rapid cell proliferation. Blockade of polyamine synthesis slows down cellular growth and proliferation of malignant tissues (29). The new drugs in the treatment of colon cancer are highly specific and non-toxic hydroxylamine-containing competitive inhibitor of ornithine decarboxylase (ODC) 1-aminooxy-3-aminopropane (APA), structural analog of ornithine (Fig. 13) and competitive inhibitor of S-adenosyl-methionine decarboxylase (SAMDC), 5'-deoxy-5'-adenosyl-methylthioethyl-hydroxylamine, (AMA) (Fig. 14), structural analog of S-adenosyl-methionine (30). This hydroxylamine - containing inhibitors of ODC and of SAMDC inhibit colon cancer cell proliferation and might be therapeutically promising in colon cancer.



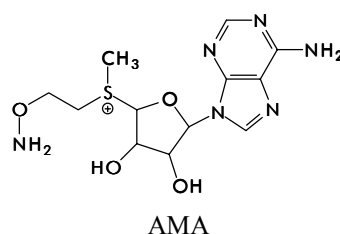
MGBG

Fig. 12.



APA

Fig. 13. 1-aminooxy-3-aminopropane



AMA

Fig. 14. 5'-deoxy-5'-adenosyl-methylthioethyl-hydroxylamine

The application of specific inhibitors of polyamine biosynthesis or degradation enables more detailed understanding of polyamine physiological role.

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KOMPETITIVNI INHIBITORI ENZIMA I NJIHOVA TERAPIJSKA PRIMENA

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Kratak sadržaj: *Enzimi katalizuju gotovo sve biohemijske procese u ćeliji. Primena mnogih lekova u medicini, antimetabolita, bazirana je na konceptu kompetitivne enzimске inhibicije. Antimetaboliti se veoma malo strukturalno razlikuju od prirodnih enzimskih supstrata. Svoja specifična dejstva ispoljavaju ponašajući se kao kompetitivni inhibitori određenih enzimskih reakcija blokirajući formiranje neželjenih metabolita u organizmu. U antimetabolite spadaju antibakterijski, antivirusni i antitumorski lekovi. Najzastupljeniji od njih jesu sulfonamidi, strukturni analozi amino kiselina (cikloserin, 5-fluoroalanin), antagonisti folne kiseline (4-amino-10-metil folna kiselina -metotreksat), analozi purina i pirimidina (6-merkaptopurin, alopurinol, 5-fluorouracil, 5-azacitidin), inhibitori biosinteze poliamina (α -difluorometil ornitin, metilglioksal-bis (guanil hidrazon=MGBG).*

Primena enzimskih inhibitora, antimetabolita, osim terapiskog značaja, omogućava bolje razumevanje raznih metaboličkih puteva, kao i bolje upoznavanje mehanizma delovanja enzima.

Ključne reči: *Enzimска inhibicija, sulfonamidi, antimetaboliti, antifolati, analozi purina i pirimidina, analozi poliamina, hemioterapija*