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(54) **Title**: THE APPLICATION OF ADENYLATE KINASE TO DISAGGREGATION AND INHIBITION OF AGGREGATION OF BLOOD PLATELETS

(57) **Abstract**: The application of adenylate kinase to inhibition of aggregation and to disaggregation of blood platelets. The adenylate kinase is from *Bacilus stearothermophilus*. The adenylate kinase is a component of medication regulating blood platelets aggregation in blood vessels and supporting the treatment of myocardial infarction, brain stroke and venous thrombosis. The adenylate kinase can also be used in prevention of myocardial infarction, brain stroke and venous thrombosis.

The application of adenylate kinase to disaggregation and inhibition of aggregation of blood platelets

The subject of this invention is the application of adenylate kinase to disaggregation of already formed blood platelets (thrombocytes) aggregates and to the inhibition of platelets aggregation.

At present two inhibitors of $P2Y_{12}$ receptor – Clopidogrel (Aprelex, Plavix, Zyllt) and Ticlopidine – are used in clinical practice to the inhibition of blood platelets aggregation. Both of them are tienopirydine derivatives and they are administered after myocardial, as well as cerebral infarction. Clopidogrel is an antithrombotic agent that lowers the blood platelets ability to create thrombus. Clopidogrel blocks the ADP-dependent mechanism of aggregation through modifying the structure of the purine receptor present on the blood platelets surface. After the first week of Clopidogrel treatment, the blood platelets activity decreases to 40-60%. Clopidogrel is applied to prevent from thrombus formation in patients after myocardial infarction or ischemic stroke and in generalized atherosclerosis. It is often used together with acetylsalicylic acid. Both of them have a similar therapeutic effect, however, they differ in mechanism of action. Their simultaneous applying is essential in some cases. Clopidogrel is a quite well tolerated medicine. The most dangerous of the eventual side effects is bleeding or haemorrhage. The medicine can also induce diarrhoea, stomach ache and indigestion. Before intended surgical intervention, the medication discontinuation should be taken into consideration, if the permanent dosage is not absolutely necessary. There are no data concerning the eventual harmful influence of Clopidogrel on the foetus and no evidence for its penetrating to breastfeeding women's milk. So it should not be used in mentioned above cases. In acute myocardial infarction the thrombolytic medicines (enzymes) like streptokinase (Streptase or tPa, Actilise, Alteptase) are used. They cause dissolving of the thrombus formed in blood vessels. Those medicines cannot be given to patients over 75 years of age, pregnant women, patients with ulcer disease. The most serious complication in the treatment is hemorrhagic stroke.

Uncontrolled aggregation of blood platelets leading to formation of thrombus is the main cause of myocardial and cerebral infarction and venous thrombosis. One of the basic signals initiating blood coagulation is adenine diphosphonucleotide (ADP). ADP is a signaling particle that activates purinergic receptors $P2X_1$, $P2Y_1$ and $P2Y_{12}$ localized on the blood platelets surface. The result of their activation are structural and biochemical changes in

thrombocytes that lead to platelets aggregation, cross-linking of the aggregates and formation of thrombus.

Adenylate kinase from *Bacilus stearothermophilus* (AKBs) has the similar Km values for both ATP and ADP as substrates. The enzyme catalyzes the reaction according to the following scheme:

$$ADP + ADP \xrightarrow{\text{adenylate kinase}} AMP + ATP$$

This mechanism of reaction catalyzed by AKBs leads to the decrease of ADP concentration with the simultaneous increase in ATP and AMP concentration. ATP is the competitive inhibitor of $P2Y_{12}$ receptor. It means that the increase of ATP concentration in blood inhibits the activity of the $P2Y_{12}$ receptor and as a result - thrombocytes aggregation. AMP, the second product of the reaction above, is degradated by 5'-nucleotidase present on the thrombocytes surface to adenosine (Ado), according to the following reaction:

$$AMP \xrightarrow{\text{5'-nucleotidase}} Ado + Pi$$

The increasing concentration of adenosine activates adenosine receptors A2a and triggers the increase in adenylate cyclase activity and cAMP concentration. It results in decreasing the calcium ions concentration within thrombocytes. These changes lead to inhibition of blood platelets aggregation.

The catalytic activity of AKBs causes the inhibition of platelets aggregation in result of three factors: eliminating of ADP present in blood, increase in ATP and Ado concentration. Therefore activity of adenylate kinase from *Bacilus stearothermophilus* decreases the blood platelets ability to aggregation and inhibits thrombus formation. The experimental results show that in the presence of AKBs the aggregation of blood platelets activated by collagen is not only stopped but also the already formed aggregates are dissolved (Tabela 1, 2).

The action of AKBs is highly beneficial. In opposite to other enzymatic preparations that hydrolyse the thrombus that is already formed, our enzyme not only prevents from blood coagulation by inhibiting the platelets aggregation but also dissolves the aggregates. As a result of its action, no thrombus is formed.

Adenylate kinase from *Bacilus stearothermophilus* can be used to the inhibition of blood coagulation in blood banks, to inhibition of uncontrolled blood coagulation in blood vessels and to reduce the disadvantageous effects of myocardial and cerebral infarction.

The analyses of deaggregative properties of adenylate kinase from *Bacilus stearothermophilus* will be described in following examples.

The platelet-rich plasma obtained from pig blood was used to experiments concerning the deaggregative function of AKBs. The platelets aggregation was initiated by 7.5μg/ml collagen. The process of platelet aggregates formation was analysed by spectrophotometric measurement at λ=600nm. Succesive samples were analysed with different amounts of adenylate kinase addded before collagen or 1 and 5 minutes after initiation of the platelets aggregation with collagen.

Patent Claims

1. A new application of adenylate kinase to disaggregation and inhibition of blood platelets aggregation.
2. The application according to claim 1, characteristic of that adenylate kinase source is from *Bacilus stearothermophilus*.
3. The application according to claim 1 or claim 2, characteristic of that adenylate kinase is a component of medication regulating the aggregation of thrombocytes in blood vessels.
4. The application according to claim 1 or claim 2, characteristic of that adenylate kinase is a component of medication supporting the treatment of acute myocardial and cerebral infarction and thrombosis.
5. The application according to claim 1 or claim 2, characteristic of that adenylate kinase is a component of medication used in preventing from myocardial and cerebral infarction and thrombosis.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KOMOSZYNSKI MICHAL ET AL: "Regulation of platelet aggregation by ecto-purine metabolizing enzymes" PURINERGIC SIGNALLING, vol. 4, no. Suppl. 1, May 2008 (2008-05), page S10, XP002601140 & PURINES 2008 MEETING; COPENHAGEN, DENMARK; JUNE 29 -JULY 02, 2008 ISSN: 1573-9538	1-5
Y	* abstract	3-5

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

International application No
PCT/PL2010/000043

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 050 007 A2 (UNITIKA LTD [JP]; RIKAGAKU KENKYUSHO [JP]; IMAHORI KAZUTOMO [JP]) 21 April 1982 (1982-04-21) the whole document	1-5

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/PL2010/000043

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