ε-AMINOCAPROIC ACID (EACA)

MAREK GACKO, ANNA WOROWSKA

Department of Vascular Surgery and Transplantation, Medical University in Bialystok
Kierownik: dr hab. n. med. M. Gacko

ε-aminocaproic acid (EACA) is aliphatic, monoamino-monocarboxylic acid. This compound was synthesised in 1899 by Gabriel and Maass (1) and its antifibrinolytic activity was demonstrated in 1948-1952 (2). The starting compound for the EACA synthesis was caprolactam. Its properties are shown in tab. 1.

Mechanism of antifibrinolytic activity

ε-aminocaproic acid is specific inhibitor of fibrinolysis. In the concentration 0.015 mg/ml, it inhibits plasminogen activation by tissue plasminogen activator (t-PA), urokinase plasminogen activator (u-PA), streptokinase (SK) and staphylokinase (SFK) (4) (fig. 1). In the concentration 6.5 mg/ml, it inhibits fibrinolytic activity of plasmin (5, 6). It does not inhibit synthetic substrate hydrolysis by plasminogen activators as well as hydrolysis synthetic substrate and protein degradation by plasmin. EACA binds to lysine binding sites (LBS) in plasminogen activators, plasminogen (free and in SK or SFK complex) and plasmin molecules (7, 8,

Fig. 1. Plasminogen activation with the use of different activators, plasmin action on various synthetic substrates and the inhibition of these processes by EACA

Arrow: continuous – activation, broken – inhibition

<table>
<thead>
<tr>
<th>Table 1. EACA characteristic (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition/property</strong></td>
</tr>
<tr>
<td>Chemical formula</td>
</tr>
<tr>
<td>Molecular weight</td>
</tr>
<tr>
<td>Melting point</td>
</tr>
<tr>
<td>H₂O solubility*</td>
</tr>
<tr>
<td>Therapeutic concentration</td>
</tr>
<tr>
<td>LD₅₀ (mouse)**</td>
</tr>
</tbody>
</table>

* - slight in ethanol, insoluble in ether and chloroform
** - intraperitoneally
Thereby EACA makes impossible the binding of these activators, plasminogen and plasmin with C-terminal lysine residues of fibrin polypeptide chains and of its soluble degradation products (fig. 2). According to the mechanism of the action, most important are ion interactions of carboxylate and amino groups of EACA with lysine binding sites of t-PA, u-PA and plasminogen/plasmin (free and in SK or SFK complex). A substitution of amino or carboxylic groups in EACA results in above 90% decrease of antifibrinolytic activity.

EACA analogues, synthetic amino acids with general formula H₂N-(CH₂)ₙ-COOH, also show antifibrinolytic activity. Their antifibrinolytic activity depends on a distance between carboxylic group carbon and amino group nitrogen. The optimal distance between these groups is 0.68 nm (10). A decrease or an increase of this distance results in a reduction or a disappearance of antifibrinolytic activity (11, 12). The ion bonds are strengthened by hydrophobic interactions between middle part of EACA with LBS. The more efficient antifibrinolytic activity of EACA analogues with the higher hydrophobicity of middle part of the structure as p-amino-methylbenzoic acid (PAMBA) and t-4-aminomethylcyclohexanocarboxylic acid (AMCA) confirm the role of these interactions (4, 13). It is illustrated by the data presented in tab. 2.

Therapeutic, indications, pharmacokinetics

EACA is used in: bleedings owing to an activation of fibrinolytic system and a overdose of t-PA or streptokinase; systemic diseases bleedings: congenital haemorrhagic diathesis, thrombocytopenia, leukaemia, aplastic anemia; surgical procedures: after heart-vascular operations, prostatectomy, lungs and liver operations and in the surgical wound bleedings; bleedings associated with internal disease: cirrhosis of the liver, oesophageal varices, chronic gastric ulcer disease; non-surgical bleedings, for example parenchymatous bleedings; obstetric-gynaecological problems: an excessive menstrual bleeding, after abortion, an excessive bleeding after placental detachment, in uterine myoma; stomatology and laryngology: prophylactic oral treatment 1 h before a tooth or tonsils extraction in the case of patients with haemophilia and locally (as tampons) after a tooth extraction or epistaxis (3, 14, 15, 16).

ε-aminocaproic acid is producing for oral taking: as a granulate drug (50%) for adults and a syrup (20%) for children and is produ-


cing the ampoules containing EACA (4 g in 20 ml) for intravenous drop infusion.

EACA is administrated orally in the dosage of 200 to 350 mg/kg body weight /24 h (divided into 6 doses) for 3-5 days. In heavy fibrinolytic states, 4 g of EACA is administrated intravenously with the use of drip infusion during 1 hour and next 1 g every hour. Total dose can not exceed 500 mg/kg body weight /24 h. \( \varepsilon \)-aminocaproic acid is absorbed almost completely from alimentary tract during 30 minutes and reaches the maximum concentration in blood after 2 hours. Therapeutic EACA concentration is 0.06-0.18 mg/ml of blood (3, 17). About 80% of EACA are excreted in the urine within 3 hours in the case of intravenous administration and within 12 hours in the case of oral administration. EACA is non-toxic in the short- and long-term use. It does not undergo changes and it is not cumulated in the organisms (18, 19, 20).

Indications and therapeutic effects of EACA were widely presented and discussed in literature (21-25), mainly as an evaluation of a decrease of postoperative bleedings and a decrease of a volume of lost blood. Therapeutic effects of EACA and aprotinine are comparable (26-32). However favourable opinions about EACA and critical voices about safety and efficiency of aprotinin application prevail.

Contraindications, interactions, undesirable effects

The use of EACA is contraindicated in the case of: thrombotic diseases of veins and arteries, artheriosclerosis with a thrombus tendency, renal diseases, blood escape into body cavities and internal organs, pathological states with a accumulation of fibrin as pneumonia, a pregnancy until the end of first trimester, a period of mother’s milk feeding, haematuria, ureters clogging by thrombus, a decrease to 1/3 twenty-four hours volume of urine, creatinine clearance lower than 80-50 ml/min (3, 14, 33, 34).

Synergistic action of EACA and peptide inhibitors has been taken into consideration (35). Hormonal replacement therapy and oral anti-conception drugs increase antifibrinolytic effect of EACA (36). Simultaneous application of these drugs may lead to thrombus formation.

Undesirable effects of EACA are rare. They can be the general ailments: a weakness, an anxiety, vertigo and headache, orthostatic hypotension, a fainting tendency, an intensive diuresis and mucocutaneous reactions (an itch, an erythema, a rash, a conjunctiva hyperaemia, a nose mucosa oedema). Intravenous administration of EACA can result in bradycardia and cardiac arrhythmia. Large doses (20 g and more daily) of EACA increase an activity of aldolase, aminotransferases, creatine phosphokinase and a concentration of potassium cation in blood. They decrease titres of isoagglutinins and anti-Rh antibodies in human plasma and inhibit kinins and C1-esterase formation (37, 38). Long-standing administration (4-6 weeks) of large doses (18-30 g daily) of EACA can result in myopathy, manifested by a muscular pain and myoglobinuria. The administration of EACA in healthy volunteers and experimental animals result in short-term increase of plasminogen, antiplasmin, and fibrinogen concentration in blood plasma (39). An impairment of platelet adhesion and aggregation and a bleeding time elongation was observed in the case of patients undergoing EACA treatment (40-43).

Determination of concentration and activity

A concentration of \( \varepsilon \)-aminocaproic acid is determined with the use of dinitrofluorobenzene (44) and ninhydrin (45). In the case of a presence of other reacting compounds in the mixture, classical chromatographic or HPLC methods of separation are used (46-49). Antifibrinolytic activity of EACA is determined after a deproteinization of plasma, urine and tissue homogenate with the use of trichloroacetic acid (TCA) or perchloric acid (HClO4) (50, 51). The purpose of this procedure is a removal of plasminogen, plasminogen activators, inhibitors of activators and antiplasmin. TCA is removed by the ether extraction and HClO4,

<table>
<thead>
<tr>
<th>Table 2. A comparison of antifibrinolytic activity of ( \varepsilon )-aminocaproic acid and synthetic cyclic amino acids (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>( \varepsilon )-aminocaproic acid (EACA)</td>
</tr>
<tr>
<td>Para-aminometylobenzoic amid (PAMBA)</td>
</tr>
<tr>
<td>Trans-4-aminomethylocyclo-heksanecarboxylic acid (AMCA)</td>
</tr>
</tbody>
</table>
is precipitated with the use of sodium hydroxide. Antifibrinolytic activity of EACA is determined by the measurement of the time of fibrinolysis using fibrinogen, plasmin and thrombin. Antifibrinolytic activity value makes possible to evaluate the EACA concentration in biological material. For this purpose, the time of fibrinolysis is measured in the presence of different EACA concentrations. The obtained results are used to plot a relationship between fibrinolysis time and a concentration. A determination of fibrinolysis time after an addition of deproteinized sample makes possible to read the EACA concentration from this chart.

In the time of euglobulin precipitation from plasma, EACA dissociates from plasminogen activators and plasminogen molecules and is not precipitated (52). Thereby it does not influence the results of measurements of euglobulin fibrinolysis time. However it influences the value of euglobulin fibrinolytic activity measured in whole plasma.

Preparative and analytical use

An association of EACA with plasminogen lysine binding sites is used to purification of this proenzyme by affinity chromatography (53). Lysine is covalent bounded to Sepharose 4B by α-amino group. Free α-carboxylic and ε-amino groups bind plasminogen molecules. Plasma is passed through Lys-Sepharose 4B column, unbounded ballast proteins are washed up by neutral isotonic solution and lysine bounded plasminogen is eluted with the use of 26 mg/ml EACA solution.

EACA is also used to the determination of fibrinogen concentration in plasma of patients with an activation of fibrinolytic system (54, 55). Blood is collected to 0.1mol/l sodium citrate containing 10 mg/ml EACA in ratio 9:1 v/v. 4.5 ml of thrombin (10 U/ml) containing 10 mg/ml EACA is added to 0.5 ml of plasma and the content of fibrinogen is determined in the isolated thrombus.

Conclusion

Literature review shows that ε-aminocaproic acid, administered intravenously and orally, showed to be an effective drug in the therapy of haemorrhagic diathesis and bleedings. It is non-toxic, non-metabolised and excreted with urine compound.

REFERENCES


Received: 30.05.2008 r.
Adress correspondence: 15-276 Białystok, ul. M. Skłodowskiej-Curie 24a