THROMBOLYSIS: NEW THERAPEUTIC STRATEGIES

Wednesday

DRUG INTERACTIONS WITH THROMBOLYTIC AND PROFLASMINOGENIC AGENTS: PHARMACOLOGIC STUDIES IN PRIMATES. J. Fared, A. Kumar, J. Walenga and D. Happensteadt. Loyola Univ., Maywood, IL 60153 USA

Thrombolytic and profibrinolytic agents have been found to exhibit varying degrees of interactions with heparin, low molecular weight heparins (LMWs), protacyclin and cyclooxgenase inhibitors. Our initial in vivo studies did not reveal significant synergistic effects between the lytic agents and the above drugs. To study the in vivo effect of these interactions a primate (Macaca mulatta) model was employed. Initially the relative fibrinolytic and/or profibrinolytic efficacies of streptokinase (SK), urokinase (Uk) and t-PA (American Diagnostica) were evaluated in normal and hypercoagulable primate (homologous serum treated) in terms of both the consumption of plasminogen, α-antiplasmin and fibrinogen, and the formation of D-dimer, FDP and D-dimer related peptides. Heparin studies, IV and SC pretreatment of the animal with a LMWH CY 222 (Choay) at 1 mg/kg, followed by administration of t-PA (5000 IU/kg IV), resulted in a marked decrease of fibrinogen and a fibrinolytic effects with a prolongation of the bleeding time. The results with CY 222 pretreatment followed by streptokinase (2500 IU/kg IV) or urokinase (2500 IU/kg IV) administration were similar but less dramatic. In this model, t-PA also showed synergistic interactions with urokinase and protein C concentrate. Pretreatment of primate with varying doses of urokinase and protein C prior to t-PA administration resulted in a marked decrease of anti-tPA titre and a slight increase in antigenic and functional t-PA. The augmentation in t-PA activity by these thrombolytic agents may be due to the expression of various regulatory fibrinogen/fibrin related peptides or the generation of endotoxin. The mechanism of LMWH induced synergism of thrombolytic agents may also involve the release of endotoxin-tPA or a reduction of rodent thromboplastin. Although preliminary, these observations suggest that thrombolytic/profibrinolytic agents exhibit varying degrees of drug interactions with themselves and with antithrombolytic agents. Preclinical knowledge of these interactions may be of value in the design of effective thrombolytic therapy or antithrombotic therapy. Furthermore, drug interactions with thrombolytic/profibrinolytic agents should be taken into account to optimize safety/efficacy of these agents on an individual basis.

DIFFERENT EFFECTS OF HEPARIN ON THROMBOLYSIS WITH t-PA AND scu-PA IN RABBITS WITH EXPERIMENTAL THROMBOSIS. J. Juhan-Vague (1), M. Stassen (2), M.C. Alema (2), J. Kreckers (2) and D. Collen (2). Laboratory of Hemostasis, GH Timone, Marseille, France (1) and Center for Thrombosis and Vascular Research, University of Leuven, Belgium (2).

Influence of heparin or low-M heparin fractions in animals with experimental thrombosis or in patients with thrombembolic disease may result in a significant reduction of the thrombus size, without being antithrombotic and with measurable changes in the blood fibrinolytic parameters.

We measured the effect of clinical grade heparin (hep) and of two low-M heparin fractions (CY216 and CY222 from Chomay, Paris, France) on thrombosis with t-PA and with scu-PA in a rabbit jugular vein thrombosis model (Collen et al., J. Clin. Invest. 71, 368, 1983). After thrombus formation, t-PA (0.25 mg/kg) or scu-PA (0.5 mg/kg) were infused over 4 hours. The heparins were administered at hourly intervals at the start and during the infusion as bolus injections of the following amounts (expressed in anti-Xa units): Hep: 70 (A) or 200 (B); CY216: 30 (A) or 90 (B); CY222: 50 (A) or 150 (B). Results were (mean ± SEM).

Thrombolysis (percent)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Controls</th>
<th>A</th>
<th>B</th>
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</thead>
<tbody>
<tr>
<td>t-PA</td>
<td>36 ± 2 (9)</td>
<td>23 ± 1 (4)</td>
<td>31 ± 2 (4)</td>
</tr>
<tr>
<td>scu-PA</td>
<td>36 ± 2 (9)</td>
<td>23 ± 1 (4)</td>
<td>31 ± 2 (4)</td>
</tr>
<tr>
<td>Anti-Xa level</td>
<td>40</td>
<td>30</td>
<td>20</td>
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It is concluded that at sufficiently high doses, heparin and to a larger extent the two low-M heparin fractions CY216 and CY222, potentiate thrombolysis by t-PA and scu-PA in this animal model of thrombosis.

CORONARY THROMBOLYSIS IN DOGS WITH A MONOCLONAL VARIATION OF HUMAN TISSUE-TYPE PLASMINOGEN ACTIVATOR LACKING THE FINGER AND GROWTH FACTOR DOMAINS. P. Gambier (1), J. M. Stassen and D. Collen. Center for Thrombosis and Vascular Research, University of Leuven, Belgium (1), Genetics Institute, Boston, MA (2) and Center for Thrombosis and Vascular Research, University of Leuven, Belgium (3).

A variant of human tissue-type plasminogen activator (t-PA-ΔFEX), with deletion of the N-terminus fibronectin-like finger (F) and epidermal growth factor (EGF) domains, and with amino acid substitution of Glu for Asn at all known N-glycosylation sites was expressed in Chinese Hamster Ovary Cells and purified to homogeneity. The thrombolytic and pharmacokinetic properties of this variant were studied in a canine model with copper-coil induced thrombosis of the left anterior descending coronary artery. Infusion of t-PA-ΔFEX at a rate of 5 μg/kg/min for 30 min in 3 dogs resulted in a plateau level in plasma of 0.66 ± 0.05 μg/ml and induced recanalization of the coronary artery within 6 ± 4 min (mean ± SEM). Bolus injections over 2 min of 0.15 mg/kg in 3 dogs resulted in peak antigen levels in plasma of 1.8 ± 0.72 μg/ml and caused reperfusion within 14 ± 6 min. Bolus injection of 0.075 μg/kg in 3 dogs gave plasma antigen levels of 0.81 ± 0.20 μg/ml and induced lysis in 31 ± 15 min. Further reduction of the bolus to 0.038 μg/kg yielded plasma peak levels of 0.43 ± 0.20 μg/ml but did not cause reperfusion within 3 hours. Bolus Injection of 0.075μg/kg of natural t-PA isolated from melanoma cell culture fluid (Mel-t-PA) resulted in plasma peak levels of 0.46 ± 0.09 μg/ml and caused recanalization in 3 hours in only 1 of 4 dogs. None of the injections was associated with systemic fibrinolytic activation and fibrinogen degradation. The disposition of t-PA-ΔFEX related antigen from plasma following bolus injection could be described by a sum of two exponential terms with t1/2a: 17 min and t1/2b: 200 min. No significant difference in disposition rates for the different bolus injections were observed. Corresponding values for t1/2a of Mel-t-PA were 3 min.

It is concluded that the deletion mutant t-PA-ΔFEX has a markedly slower disposition rate from plasma than intact t-PA, which renders it relatively more effective than natural t-PA after bolus injection.