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FLUORESCENCE STUDIES ON THE MODE OF ACTION OF TWO SYNTHETIC THROMBIN INHIBITORS, NO. 205 AND NO. 805. S. Nagano, S. Okamoto, K. Ikezawa, K. Mimura, A. Matsuoka, A. Hijikata and Y. Tamao. Hyogo College of Med., Kobe Univ. School of Med. and Central Res. Lab., Mitsubishi Chem. Ind.

Among a novel series of synthetic thrombin-inhibitors belonging to arginine derivatives found by S. Okamoto et al., No. 205, dansyl-arginine-piperidineamide, was reported by Mann and his associates to show the enhancement of fluorescence intensity when bound to thrombin. However, No. 205 was reported by S. Okamoto et al. to inhibit pseudocholinesterase with very low Ki also, while No. 805, (4R-methyl-[methyl-tetrahydro-quinolinesulfonyl-arginyl-] 2R-piperidinecarboxylic acid), showed practically no inhibitory action on pseudocholinesterase remaining very potent inhibitory action to thrombin with a Ki of 0.019 μM. Therefore, comparative studies of fluorescence variation with No. 205 and No. 805 were made using thrombin, pseudocholinesterase and truecholinesterase as the target enzymes and the following results were obtained. 1) The enhancement of fluorescence intensity of No. 205 with thrombin was comfirmed but the same order enhancement of fluorescence intensity of No. 205 was also observed with pseudocholinesterase, but not with truecholinesterase (to this enzyme Ki of No. 205 was large), indicating that the enhancement is concomitant with the inhibitory binding of No. 205 with the enzymes. 2) The enhancement of fluorescence intensity of No. 205 with thrombin was clearly reversed by the addition of No. 805, indicating the replacement of No. 205 bound to the thrombin by No. 805. This supports the possibility of the fluorometric differentiation of thrombin from pseudocholinesterase, which allows us to discuss the histochemical application of synthetic thrombin inhibitors to demonstrate the local thrombin.

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INHIBITORY EFFECT OF GABEXATE MESILATE ON EXPERIMENTAL DISSEMINATED INTRAVASCULAR COAGULATION. H. Ohno, S.W. Chang J. Kambayashi, S. Imaoka* and G. Kōsaki. The Second Department of Surgery, Osaka University Medical School and *Department of Surgery, Center for Adult Dis.,Osaka, Japan.

Gabexate mesilate:[Ethyl p-(6-guanidinohexanoyloxy)benzoate]methanesulfonate, which is clinically used as a potent trypsin inhibitor in Japan, inhibits competitively the hydrolytic reactions with synthetic substrates by thrombin and factor Xa. However, its virtual anticoagulant effect in vivo remains to be evaluated, because of its short half-life due to the presence of plasma esterase. In the present study, the inhibitory effect of gabexate mesilate on experimental disseminated intravascular coagulation was investigated in comparison with those of heparin and aprotinin.

^{125}I-Fibrinogen was injected into rats along with t-AMCHA to prevent lysis of fibrin formed. Five minutes after a infusion of gabexate mesilate, heparin, aprotinin or saline, intravascular cogulation was triggered by a continuous infusion of thrombin or tissue thromboplstin. The deposition of fibrin in kidney was continuously monitored by scintillation detector. Platelet count and ^{125}I-fibrinogen in blood were determined. The formation of fibrin induced by thrombin and tissue thromboplastin was completely inhibited by continuous administration of 120 μmol gabexate mesilate/kg/h or 200 U heparin/kg/h. On the other hand, aprotinin failed to prevent the deposition of fibrin even at dose of 50,000 U/kg/h. Also, other parameters corroborated the same aspect. The naturally occuring lysis of the microthrombi previously formed by thrombin was not blocked by the infusion of 120 μmol gabexate mesilate/kg/h or 200 U heparin/kg/h. However, aprotinin, at 5,000 U/kg/h, completely blocked the lysis. Using clotting assay, the biological half life of gabexate mesilate in human plasma was found to be ten times longer than that in rat plasma, suggesting that much lower dose would be effective in human. In conclusion, gabexate mesilate is a relevant compound as an antithrombotic agent.

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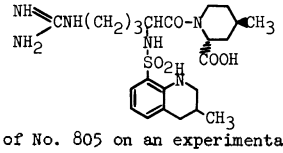
THE EFFECT OF THE SYNTHETIC THROMBIN INHIBITOR, No. 805, ON AN EXPERIMENTAL DIC IN RABBITS. Y. Tamao, H. Hara, R. Kikumoto and *S. Okamoto. Mitsubishi Chemical Industries Ltd., Midori-ku, Yokohama, Japan and *Kobe University School of Medicine, Ikuta-ku, Kobe, Japan.

Our studies of synthetic thrombin inhibitors of arginine derivatives yielded very recently No. 805 (MCI-9038 or MD-805) having a specific stereostructure of 4-methyl-2-piperidinecarboxylic acid at its carboxamide portion which showed an extremely potent and selective inhibition of thrombin with a Ki of 0.019 μM.



(No. 805)

The effect of No. 805 on an experimental DIC was studied. An experimental DIC in rabbits was generated by a constant i.v. infusion of 0.1 M lactic acid and crude bovine placental tissue thromboplastin. A rapid decrease in platelet number and a gradual decrease in fibrinogen content in plasma were observed over the experiment period of 5 hrs. The i.v. infusion of No. 805 suppressed the decrease in platelet number and fibrinogen content dose-dependently from 1.0 to 31.6 μg/kg/min and the suppression of ca. 50% was attained at a dose of 3.16 μg/kg/min showing a plasma level as low as ca. 0.3 μM, while heparin given at a dose of 100 or 1,000 u/kg i.v. (x2) did not show a notable suppression of the platelet number decrease but a significant suppression of the fibrinogen content decrease. Thus, No. 805 was shown to be effective on the experimental DIC at lower plasma level, exhibiting more dominant effects on platelets than heparin. This result was carefully compared with those on other animal thrombosis.

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INTERACTION OF PROTAMINE SULFATE WITH THROMBIN. R. Cobel-Geard, J.A. Penner, H.I. Hassouna. Dept of Medicine, College of Human Medicine, Michigan State University, E. Lansing, Michigan, U.S.A.

Protamine sulfate, a simple protein M.W. 4,600, is a known antiheparin agent which, in the absence of heparin, demonstrates an anticoagulant activity both in vitro and in vivo. Much work has been done to elucidate the interactions of heparin with thrombin and ATIII, however, little is known about the mechanism of protamine sulfate anticoagulant activity and its effect on thrombin.

In binding studies, radiolabeled thrombin was reacted with protamine for 1 hour and ATIII was then used as a probe to test the availability of the thrombin active site. ATIII/*thrombin complex was separated from free *thrombin on a Sephacryl S200 molecular sieve column. As a result of preincubating thrombin with protamine, ATIII was unable to bind thrombin. Functional assays for thrombin activity were carried out in a system where time taken for formation of a thrombin catalyzed fibrin clot was the measure used for thrombin activity. Effect of protamine on thrombin activity when tested in this system demonstrated that with increasing molar concentrations of protamine, there is a corresponding decrease in thrombin activity. Maximum inhibition of activity occurred at a protamine concentration of 200 μM. This inhibition was concentration dependent, partial and reversible. In contrast the amydolysis of the synthetic substrate H-D-Phe-Pip-Arg-p-nitroanilide by thrombin was unaffected by protamine.

Results of these studies have confirmed the anticoagulant role of protamine sulfate and provided information on its interaction with purified thrombin. Since the drug did not totally inhibit functional activity of thrombin, we feel that it binds the enzyme, partially blocking the active site.

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