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An amidrazonophenylalanine derivative, LB30057, inhibits the catalytic activity of thrombin potently by interaction with active site of thrombin (Ki=0.38 nM), and has higher water solubility. However, the effect of LB30057 on the biological activities of thrombin at various tissues has not been investigated. The aims of this study were to evaluate the effect of LB30057 on the biological activities of thrombin at various tissues, and to determine whether thrombin inhibition by LB30057 could reduce the incidence of occlusive thrombosis in in vivo model. In human plasma, LB30057 prolonged plasma coagulation time in a concentration-dependent manner, as determined by activated partial thromboplastin time, prothrombin time, and thrombin time. In human platelets, thrombin-induced platelet aggregation was inhibited by LB30057 potently with an IC50 of 0.0015 mM and 0.0546 mM for washed platelets and platelet rich plasma, respectively. In contrast, much higher concentrations of LB30057 were required to inhibit other agonist-induced platelet aggregation. LB30057 showed the inhibitory effects on the serotonin secretion (IC50=0.0015 mM), the P-selectin expression (IC50=0.0025 mM), and the increase of intracellular calcium level (IC50=0.020 mM) induced by thrombin in concentration-dependent manners. However, the treatment with LB30057 to platelets did not induce any cytotoxicity, as determined by the release of lactate dehydrogenase. In the blood vessel isolated from guinea pig, the treatment of LB30057 resulted in the concentration-dependent inhibition of thrombin-induced vascular contraction. In vivo study revealed that LB30057 following oral administration significantly reduced the incidence of occlusion and improved carotid arterial patency using the FeCl3-induced carotid artery thrombosis model in rats. All these results suggest that LB30057 is a potent inhibitor of the biological activities of thrombin at various target tissues and, thus, might be developed as an antithrombotic agent for treatment and prevention of thrombotic diseases.

[OA-3] [04/20/2001 (Fri) 14:00 - 14:15 / Room 1]

Effect of DA-9601 on cerulein-induced pancreatic fibrosis: In vitro and in vivo effect

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Since pancreatic fibrosis is a severe complication in pancreatic diseases such as chronic pancreatitis and pancreatic cancer, it is of importance to establish an appropriate model with which to study this fibrotic process. However, unfortunately there are only few studies dealing with the development and persistance of pancreatic fibosis. In the present study, repetitive induction of acute cerulein-pancreatitis led to fibrosis fo the pancreas in mice. Methods: We injected cerulein (50mg/Kg) 6 times every hour twice per week subcutaneously and continued to inject cerulein for 10 weeks. Results: Atrophy, trans-differentiation of acinar units to duct like tubular complexes, islets hyperplasia, and dilatation of intraacinar lumina developed. Masson-trichromestaining demonstrated progessive accumulation of extracellular matrix in interlobar and interacinar spaces. a-smooth muscle actins, fibronectins, collagen I were prominently accumulated as time passed. We treated ICR mice with DA-9601, phytophamaceuticals possessing antioxidative effects, 100 mg/Kg and compared the degree of pancreatic fibrosis between repeated cerulein alone group and DA-9601 + cerulein group. There was a statistically significant difference in the score of pancreatic fibrosis between these two groups (2.63 ±0.44 vs. 1.63±0.28, $p<0.01$). Using nuclear extracts of pancreas and radiolabeled NF-kB probe, EMSA was performed showing significantly increased mobility shift assay of cerulein-induced pancreatitis group and marked attenuation of DA-9601 treated group. p65 subunit of NF-kB was majorly binding protein identified by supershift EMSA. Pancreatic stellate cells (PSC) were isolated from fibrotic pancreatic tissues. Activations of PSC were attenuated by DA-9601 treatment. Conclusion: We could establish the mouse model of chronic pancreatitis by repeated cerulein administration and DA-9601 showed excellent preventive effect suggesting clinical trial.

[OA-4] [04/20/2001 (Fri) 14:15 - 14:30 / Room 1]