Short Communication

Changes in Blood Viscosity With Synthetic Protease Inhibitors

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Received December 11, 2002; Accepted February 10, 2003

Abstract. We examined the effects on whole blood viscosity and coagulation time of various dosages of the synthetic low-molecular protease inhibitors gabexate mesilate and nafamostat mesilate with an oscillation-type viscometer. When either agent was added, blood viscosity decreased dose-dependently along a sigmoid-like curve. Furthermore, coagulation time was shorter with gabexate mesilate than with nafamostat mesilate owing to the differences of half-life in human blood. Thrombin generation, which results from the activation of coagulation factors, is inhibited by synthetic protease inhibitors and subsequently decreases blood viscosity dose-dependently.

Keywords: blood viscosity, synthetic protease inhibitor, rheology

Gabexate mesilate (GM) and nafamostat mesilate (NM) are synthetic, low-molecular-weight, protease-inhibiting drugs. They inhibit trypsin-like serine proteases, such as the activated coagulation factors II, VII, IX, X, XI, and XII, and plasma kallikrein (1-4). These agents are effective for the treatment of disseminated intravascular coagulation, a pathologic syndrome in which fibrin thrombi are formed, and acute pancreatitis (1, 3, 5). Furthermore, GM is used as a regional anticoagulant in hemodialysis (6, 7). However, to our knowledge, the relation between blood viscosity and these synthetic protease inhibitors has not been examined. In this study, we examined the dose-dependent effects of GM and NM on whole-blood viscosity (BV) and coagulation time.

Blood samples were drawn from a cubital vein of healthy male volunteers (aged 20 to 22 years), none of whom were obese or were smokers, between 9 and 11 a.m. Informed consent was obtained from all volunteers. Thirty microliters of GM or NM was added to a 3-ml sample of blood and gently mixed by inversion. GM was obtained from Ono Pharmaceutical Co. (Osaka) and prepared to adjust the final concentration of GM to 1.0×10⁻⁴, 4.0×10⁻⁴, 8.0×10⁻⁴, or 1.0×10⁻³ M. NM was kindly provided by Torii Pharmaceutical Co. (Tokyo) and prepared to adjust the final concentration of NM to 1.0×10⁻⁶, 5.0×10⁻⁶, 1.0×10⁻⁵, or 5.0×10⁻⁵ M. As a control, normal saline was added instead of the drugs to the blood samples. Four to five samples were examined at each concentration, including the control.

Immediately after a synthetic protease inhibitor or saline was mixed with a blood sample by inversion, the mixed blood was gently poured into a bottle incubated at 33°C, the same temperature as peripheral blood. BV was determined immediately with an oscillation viscometer (Viscomate VM-1G; Yamaichi Electronics, Osaka). As the probe of the viscometer, made from titanium, oscillates at 500 Hz, the BV can be measured at a shear rate of 400 to 500 per second. This system developed by us can be used to examine changes in BV over time and estimate the action of the coagulation phase (8, 9). Generally, BV is controlled by hematocrit, plasma viscosity, red blood cell deformability, and red blood cell aggregation (10). When the shear rate is raised above 200 per second, the aggregates of red blood cells are completely dispersed with higher deformation (11). Therefore, as BV is nearly constant at high shear rate, we can adequately measure BV in this method. Furthermore, the blood was analyzed immediately without addition of anticoagulants, so BV and coagulation...
time could be determined with great accuracy. In addition, the blood samples were used to estimate hematocrit immediately by centrifugation of the capillary tubes at 12,000 rpm for 5 min in a hematocrit centrifuge.

The BV of untreated blood was almost constant (4.2 ± 0.2 mPa · s) when the hematocrit was nearly constant (45.9 ± 1.5%) among the volunteers (n = 24). In control blood samples, BV reached a plateau phase lasting approximately 180 s, and then showed a marked increase, which reflects the progression of the coagulation cascade and subsequent clot formation. The time courses of changes in BV when various concentrations of GM and NM had been added are shown in Fig. 1 and 2. With higher concentrations of these agents, BV in the plateau phase decreased and coagulation time increased. However, BV increased faster with GM than with NM, a difference that reflects the shorter coagulation time with GM. The lowest values of BV were plotted according to the concentration of each agent (Fig. 3). With GM, BV decreased in a dose-dependent manner along a sigmoid-like curve from 4.1 ± 0.1 mPa · s in untreated blood to a minimum of 2.6 ± 0.3 mPa · s (63.4% of that in untreated blood) at a concentration of 1.0 × 10⁻³ M. With NM, BV decreased in a dose-dependent manner along a sigmoid-like curve from 4.2 ± 0.2 mPa · s in untreated blood to a minimum of 1.9 ± 0.2 mPa · s (45.2% of that in untreated blood) at a concentration of 5.0 × 10⁻⁵ M. BV decreased markedly with GM concentrations of 4.0 × 10⁻⁴ to 8.0 × 10⁻⁴ M and with NM concentrations of 5.0 × 10⁻⁶ to 1.0 × 10⁻⁵ M.

Blood coagulation is due to successive activation of proenzymes to specific proteolytic enzymes. The sequence of enzyme reactions involves biologic amplification and feedback mechanisms. Our experiment, examination of BV over time, estimates the cascade reaction of coagulation and subsequent fibrin formation. Because thrombin is generated by prothrombin at the end of a series of events, thrombin plays a pivotal role in the regulation of hemostasis and thrombosis (12). As we have previously found that antithrombotic agents decrease BV dose-dependently, the drugs influencing a series of coagulation systems affected BV (8). In this study, we examined the effect on BV of synthetic protease inhibitors, specifically, inhibitors of activated coagulation factors.

The blood coagulation system comprises intrinsic and extrinsic pathways; the intrinsic pathway is initiated by activation of factor XII on a negatively charged surface, and the extrinsic pathway is initiated by binding of factor VII to tissue factors derived from injured tissue, activated monocytes, or endothelial cells (12). Although GM potently inhibits factor Xa, thrombin, plasma kallikrein, and plasmin, which are proteases involved in the coagulation and fibrinolysis system, NM also inhibi-
its factor XIIa and the extrinsic pathway of coagulation by inhibiting the tissue factor-factor VIIa complex (3, 4, 6, 13, 14). Although we observed a similar phenomenon in the present study that both GM and NM decreased BV in a dose-dependent manner along a sigmoid-like curve, NM decreased BV some 100-fold more strongly than did GM at the same concentration. These differences between GM and NM may be due to NM’s inhibition of the early components of the intrinsic pathway, such as factor Xa, and of the extrinsic pathway. Our results also demonstrate a difference in coagulation time: BV rose faster with GM than with NM. A possible reason for this difference is a difference in stability in human blood. GM is rapidly metabolized by plasma elastases and has a half-life of 55 s; in contrast, NM has a half-life of 23.1 min (14, 15). Previous reports have shown that GM reaches a concentration in human blood of $6.3 \times 10^{-7}$ M after being administrated at 4 mg/kg over 1 h, and that of NM reaches a concentration of less than $1.1 \times 10^{-7}$ M after being injected at 20 mg/kg over 1 h. Therefore, our results suggest that the changes in NM and GM concentration within the range of doses used clinically cause little change in BV (14, 15).

Because thrombin generation results from the activation of coagulation factors, it is inhibited by the synthetic protease inhibitors. To our knowledge, this is the first report to show the relation between BV, an index of thrombotic tendency and the synthetic protease inhibitors. Although the detail mechanisms of these phenomena may be clarified by further studies, our results suggest that high doses of synthetic protease inhibitors decrease BV dose-dependently.

The main findings of this study were presented at the 24th Congress of the Japanese Society of Biorheology, Yokohama, June 2001.

References


