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Background: Heparins are sulphated glycosaminoglycans mainly used as an anticoagulant therapy to prevent venous thromboembolism. However, anticoagulants as heparinoids are known to impact coagulation tests by increasing clotting times or reducing thrombin generation in the test sample. Therefore, to ensure an unbiased evaluation of the patient coagulability status, neutralization of the anticoagulant effect of heparinoids is necessary. This can be achieved by using neutralizing agents such as hexadimethrine bromide, also known as polybrene, or Hepa-remove® (5-Diagnostics, Switzerland), a newly developed synthetic tripeptide.

Aims: This study aimed to assess the capacity of polybrene and Hepa-remove® to neutralize the anticoagulant effect of enoxaparin on thrombin generation assay (TGA) and to evaluate their impact on this test.

Method: Low molecular weight heparin was spiked in a normal pooled plasma composed of 50 healthy individuals at different concentrations, 0 [=sterile saline solution], 0.2, 0.4 and 0.6 UI/mL. TGA was assessed on the ST Genesia system using STG-ThromboScreen -TM (Stago, France) as triggering reagent. Polybrene and Hepa-remove® were added into the triggering reagent upon reconstitution in order to obtain final plasma concentrations of 0.025mg/mL and 0.166mg/mL, respectively. Analyses were performed in duplicate and assessed in three independent runs. The impact of these neutralizing agents on TG was assessed by comparing plasma (without heparin) treated with and without Polybrene or Hepa-remove®. The neutralizing capacity was measured as the percentages of change from baseline in which the plasma without heparin treated with Polybrene or Hepa-remove® was considered as the baseline condition.

Results: No TGA parameter was impacted by the presence of polybrene at the concentration tested in this study. On the opposite, Hepa-remove® significantly reduces he Lag Time (LT) and the Time-to-peak (Ttp) (p-*value*< 0.05). Polybrene permits to fully neutralize the effect of enoxaparin. The residual Endogenous Thrombin Potential (ETP), mean Velocity Rate Index (mVRI) and Peak Height (PH) after treatment by polybrene were increased by 5.1%, 8.6% and 6.6% compared to baseline TG on NPP spiked with saline, even at the highest enoxaparin concentration tested (i.e., 0.6 IU/mL). The LT decreased by 6.4%. On the other hand, the Ttp decreased by 3.9% which was significant compared to the baseline (p-*value* = 0.048). The Hepa-remove® permitted the recovery of the baseline ETP, mVRI and PH values regardless of the heparin concentration. However, as it impacted the LT and Ttp even in absence of heparin, it does not permit to recover the baseline value of these parameters in absence of enoxaparin.

Conclusion: This study showed that polybrene had no impact on TG under our assay conditions; while the Hepa-remove® impacted the LT and Ttp. Regarding their neutralizing capacity, polybrene was able to fully neutralize enoxaparin up to a concentration of 0.6 UI/mL with no impact on TG. The Hepa-remove® showed acceptable neutralizing capacity, but impacted some TG parameters independently to its neutralizing capacity on enoxaparin.