UNIVERSITY OF BELGRADE
FACULTY OF PHARMACY

SERUM DETERMINATION OF 99M TECHNETIUM RADIOLABELED TIROFIBAN USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN THE ANIMAL RAT MODEL OF INTRODUCED ACUTE DEEP VENOUS THROMBOSIS

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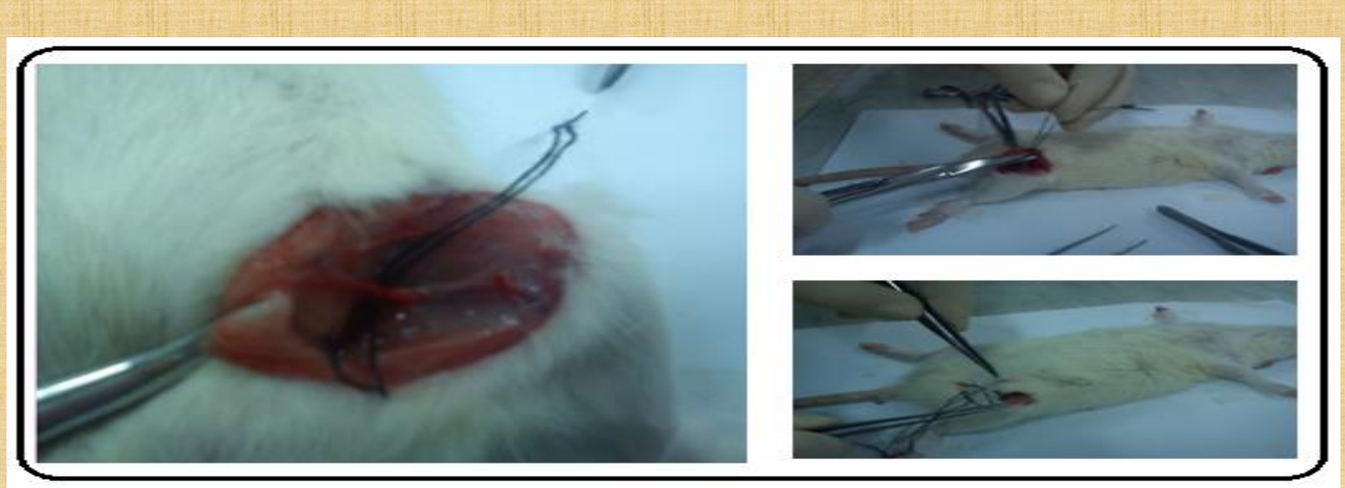
INTRODUCTION

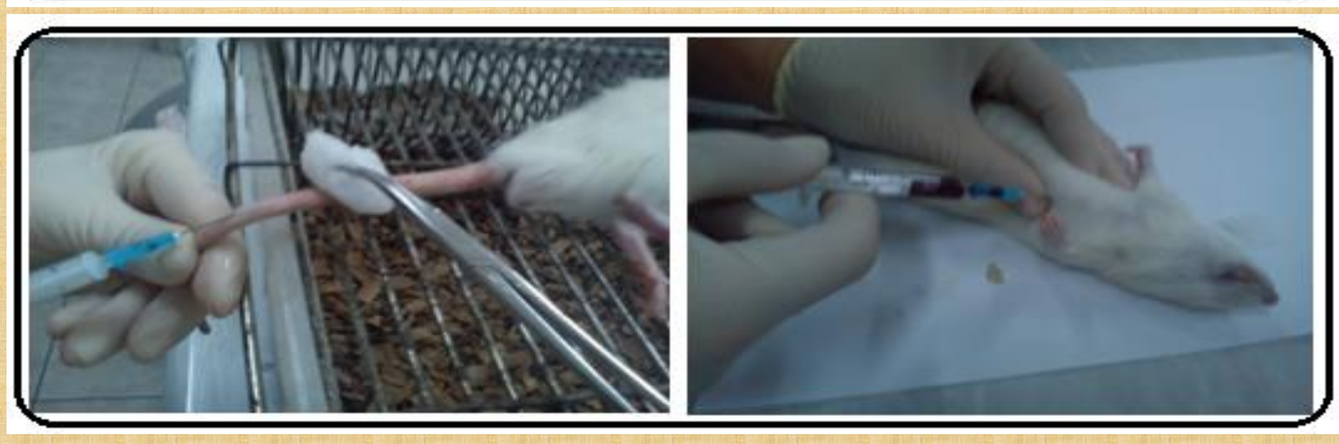
The development of radiolabeled small peptide or peptidomimetic ligands can bind platelets and their specific expressed receptor have been suggested as a new approach to detect the clot location and, more essentially, to determine the age and morphology of the evolving thrombus. This new approach is focused on the use of a series of radiolabeled platelet GPIIb/IIIa receptor antagonists.
Tirofiban N-(butylsulfonyl)- 4-O-(4-(4-piperidyl)-L-tyrosine is a non-peptide tyrosine derivate.

The aim of the study was to introduce radioactive-labeled tirofiban as a specific imaging agent for acute DVT and to determine the serum concentrations in normotensive male Wister rats with and without deep acute venous thrombosis in order to confirm the animal model of acute venous thrombosis.

MATERIAL AND METHODS:

Venous thrombosis was induced by ligature of the femoral vein in rats whose blood was made hypercoagulable by intravenous administration of tissue thrombin. The determination of Tirofiban in serum was performed using validated HPLC method with UV detection.

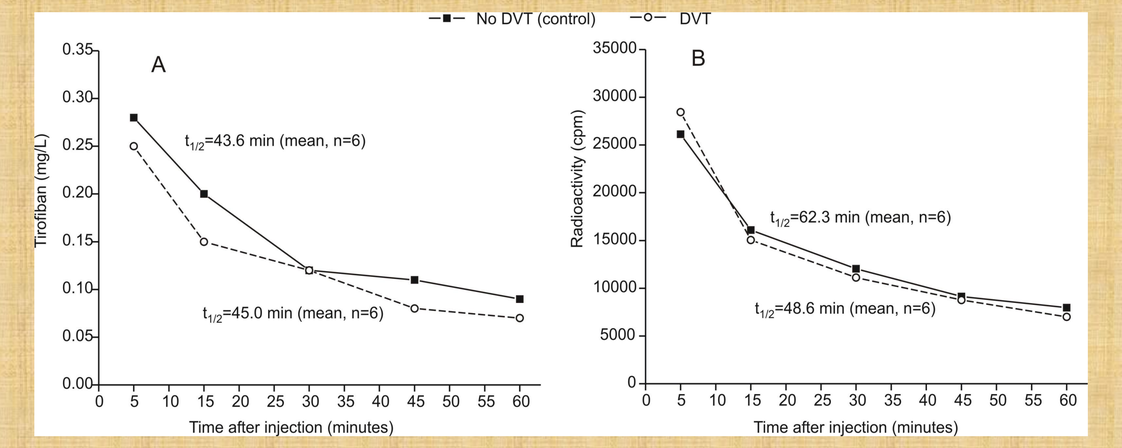




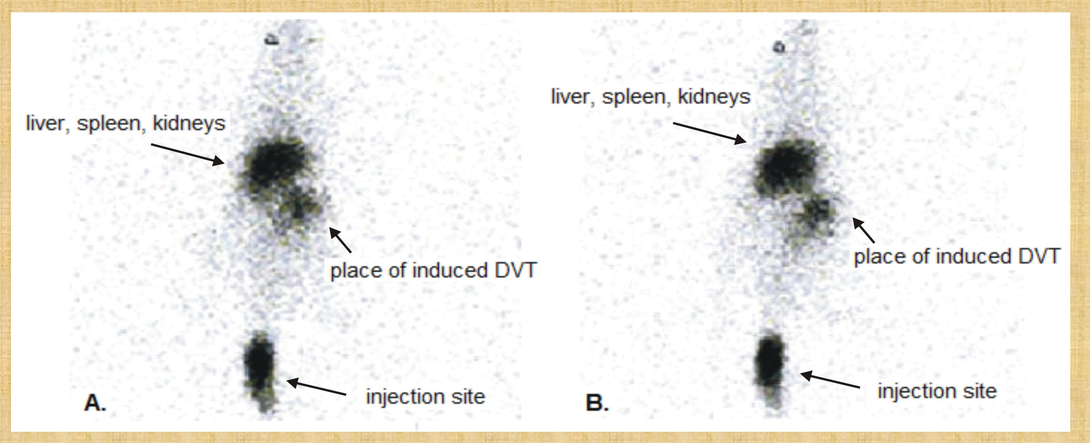
Rats (n=6 per group) were injected (tail vein) with unlabeled tirofiban (0.6 mg/kg) and with 99mTc-tirofiban (3-4x105 cpm, corresponding to 2 nmol of tirofiban).

RESULTS AND DISCUSSION

The labeling was performed with technetium-99 in the presence of a stannous reducing agent and biodistribution and visualization of the labeled molecule was carried out using the same experimental model of DVT.
The serum concentrations of Tirofiban measured after 5, 15, 30, 45 and 60 min in the group of rats with DVT were lower as compared to the serum concentrations of Tirofiban in the control group of rats. During the determination of the serum concentration planar imaging was performed at 30 and 60 min after application.
These values were considered positive in the detection of acute DVT and corresponding to values of serum application obtained from the normal rat and experimental model.



Mean serum concentrations of unlabeled (A) and of 99mTc-tirofiban (B) over 60 minutes after intravenous injection in rats without or with induced deep venous thrombosis (DVT).



Visualization of distribution of 99mTc-Tirofiban and its binding to the critical areas in experimental rat model with DVT

CONCLUSION

The high DVT uptake and lower serum concentrations of Tirofiban measured in the group of rats with DVT shows that radiolabeled tirofiban in the introduced rat model can be a promising agent for imaging the deep venous thrombosis.