Novel MRI Contrast Agent for Thrombus Detection

Y. Kawata[1], Q. Han[2], H. Schmitt-Willich[3], K. Yasugi[1], T. Tsuji[4], N. Tsuda[4], T. Yokawa[1], H. Weinmann[3]

[1]Drug Discovery Institute, Nihon Schering K.K., Osaka, Osaka, Japan, [2]Pharmaceutical Development, Nihon Schering K.K., Osaka, Osaka, Japan, [3]Research Laboratories, Schering AG, Berlin, Berlin, Germany, [4]Preclinical Development, Nihon Schering K.K., Osaka, Osaka, Japan

Synopsis

A macrocyclic gadolinium chelate linked to the small peptide Gly-Pro-Arg-Pro-Pro was investigated as a thrombus imaging MR contrast agent. This peptide should bind to a specific region of fibrin/fibrinogen. The human fibrinogen binding of the gadolinium chelate was ca. 70% while the albumin binding was ca. 10%. The concentration of the gadolinium chelate in the thrombus was seven times higher than in blood 3 hours after intravenous injection in photochemically induced thrombosis rat model. The influence on the blood coagulation (bleeding time) was negligible.

Introduction

There is no golden standard to diagnose deep vein thrombus (DVT) at the moment although X-ray venography, US and D-dimer test are widely used in clinically routine. In the clinical diagnostic imaging methods, thrombus is observed indirectly due to a signal void of the lesion in the blood vessel. To detect the thrombus more accurately, it is expected to detect the thrombus directly. Recently radiolabeling contrast media have been investigated [1)].

The purpose of this study was to enhance a blood thrombus by a newly developed gadolinium (Gd) compound. Synthetic peptide Gly-Pro-Arg-Pro and Gly-Pro-Arg were reported as anti-thrombi agents [2)] bind to the specific site near D-domain of fibrin (C-termini of fibrin γ) due to their amino acid sequences analogous to N-termini of fibrin α chain and prevent the formation of fibrin clotting. In addition, Gly-Pro-Arg-Pro-Pro was reported as an amino acid sequence with much more high affinity to the specific site of fibrin than Gly-Pro-Arg-Pro or Gly-Pro-Arg-Val-Val [3)].

1 was newly synthesized to bind to fibrin; a macrocyclic Gd chelate combined with a small peptide Gly-Pro-Arg-Pro-Pro (Fig. 1). The binding to fibrinogen and fibrin, its T1 relaxivity, clot distribution and bleeding time were investigated to evaluate **1** as a potential thrombus imaging MR contrast agent.

Methods

1. Materials; **1** was newly synthesized. Fibrinogen from human and other species, thrombin and rosebengal were purchased from SIGMA, Dade Behring and Wako, respectively. Gd-DTPA (Magnevist) was used as a reference.
2. Protein binding test : [Fibrinogen binding] **1** was diluted to 1 mmolGd/L in 3 % of fibrinogen buffer solution (pH ca. 7) and incubated at room temperature for 15 min. Each solution was ultrafiltrated with Centrisart-C4 (Mw 10,000, Sartrius) and Gd concentrations in the filtrates and the fibrinogen solutions were determined by ICP. [Fibrin binding] Fibrinogen solution and thrombin solution from human were mixed and incubated to coagulate. **1** was added to the formed fibrin gel and incubated at room temperature over night. The supernatant on the fibrin gel was removed and the unbound **1** to the fibrin gel was separated from the fibrin gel by ultrafiltration (Centriplus 100, Amicon, cut off: 200,000). The Gd concentrations in the fibrin gel and the supernatant were determined by ICP.
3. T1 relaxivity measurements; **1** was dissolved in buffer or protein solutions to 0-1 mmolGd/L. T1 relaxation times of the solutions were measured by means of an NMR analyzer (Minispec PC120, Bruker 0.47T) at 37℃.
4. Preparation of Photochemically Induced Thrombosis (PIT) model rats : Wistar rats (male, 357-408g) were studied under anesthesia with pentobarbital. 20mg/kg of rose bengal was intravenously injected and the femoral vein was irradiated with xenon light (540 nm) for 20 min.
5. Clot distribution; 0.05 mmolGd/kg of **1** was intravenously injected in PIT rat model and the Gd concentrations in the thrombus and in the blood were measured 3 hours after the administration by ICP.
6. Bleeding time; Bleeding time from tail vein was measured in wistar rats (male/femal=5/5, 165-183g) at 37℃ after intravenous injection (0.025mmolGd/kg) of **1**. Saline was injected as a control.

Results and Discussion

The new contrast agent exhibits a specific binding to human and rat fibrinogen. A binding of ca. 70 % was calculated while human albumin binding was ca. 10 %. The binding rates to human fibrinogen and to rat fibrinogen were 71% and 70%, respectively. The values were larger than that to albumin of 11%, to bovine fibrinogen of 59%, to sheep fibrinogen of 53% and to porcine fibrinogen of 46%. On the other hand, there was almost no affinity to fibrin gel. The amino acid sequence (Gly-Pro-Arg-Pro-Pro) has high affinity to the specific site of C-termini of fibrin γ chain and the specific site is occupied by N-termini in fibrin α chain after forming fibrin gel, thus **1** had almost no affinity to the fibrin gel formed completely with thrombin. T1 proton relaxivity of **1** in water was ca. 6 L/mmol*s and it slightly increased when bound to fibrinogen. The increase of the relaxivity was not comparable to the increase of the relaxivity of other paramagnetic agents bound to albumin.

Gd concentration in the thrombus (56 nmolGd/g) was higher than in the blood (8 nmolGd/mL) 3 hours after the intravenous injection of **1**.

Since bleeding is one of the adverse reactions of DVT treatment, bleeding time was measured after intravenous injection of **1**. Bleeding time was ca.160 sec while the control was ca. 130 sec. The prolongation of the bleeding time was negligible since commercially available iodine X-ray contrast media elicit stronger effects.

In conclusion, the newly synthesized macrocyclic Gd contrast agent binds specifically to fibrinogen or blood clot, in which C-termini of fibrinogen γ or fibrin γ chain still remains, and eliminated rapidly without significant prolongation of the bleeding time. This type of Gd chelates is worthy of further study.

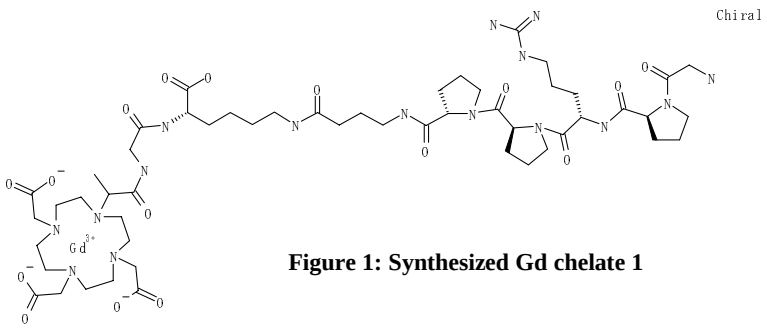

Figure 1: Synthesized Gd chelate 1

References

[1] Thakur, M.L. et al. J Nucl. Med. 41 (1) 161, 2000
[2] Laudano, A.P., Proc. Natl. Acad. Sci. USA, 75, 7, 3085, 1978
[3] Kawasaki, K., Chem. Pharm. Bull., 41(5), 975, 1993