

PROJECT TITLE: Thrombolysis by sonication

AIMS: To improve the role of thrombolytic treatment in the clinic by enhanced action and diminishing side effects

PROJECT SUMMARY: Since thrombolytic treatment is accompanied by the risk of bleeding, the use of ultrasound enhanced fibrinolysis is a way of increasing the efficacy of fibrinolytic therapy. The development of non-invasive ultrasound enhancement of thrombolytic treatment started in the early 1990s. The Department of Cardiology in Lund was one of the early participants [1, 2]. During the 1990s, many *in vitro* and *in vivo* investigations showed that ultrasound enhanced treatment of fibrinolysis worked. The development and research in the field performed at the Department of Cardiology in Lund has focused the work in the high frequency area (1 MHz). Results from our own recent studies have clarified many of the mechanisms involved in the field of high frequency ultrasound enhanced fibrinolysis. In one study it we showed that exposure of the streptokinase molecule to ultrasound modulates its fibrinolytic properties, resulting in both increased and decreased fibrinolytic effects. These effects were present during simultaneous exposure of the clot and the streptokinase solution as well as after pre-exposure of the streptokinase solution suggesting a direct effect of ultrasound on the streptokinase molecule [3].

In a second study it was shown that pre-exposure of the reteplase solution to low intensity during one hour induced changes in the function of the molecule associated with enhanced fibrinolytic effects in the following fibrinolytic evaluation compared to unexposed reteplase solution. Enhancement effects occurred also when clots were exposed to high intensity of ultrasound before or concomitantly to exposure to reteplase. This suggests two different intensity-dependent mechanisms involved during ultrasound enhanced reteplase fibrinolysis [4]. We have also performed a pilot-study using micro-bubbles in combination with ultrasound with the intention for use in the setting of ultrasound enhanced fibrinolysis. In parallel with the development of the method we have conducted studies of the safety using this ultrasound exposure on ischemic organs [5, 6]. This in combination with earlier studies in the same field from the Department of Cardiology [1, 2, 7-11] we think we can reach a clinical useful method in nearby future. However, to further optimize the method of improving thrombolytic drugs, it is of importance to do additional *in-vitro* investigations of the optimal ultrasound configuration. We aim at doing:

- Exploration of the optimal frequency, in the range between 500 kHz to 4 MHz.
- Exploration of the optimal number of pulses of ultrasound needed for optimal effect (10 pulses (p)/ms, 20p/ms, 50p/ms 100p/ms, 500p/ms, 1000p/ms).
- Exploration of the optimal exposure time needed for enhanced effects of the thrombolytic drugs; exposure times have so far been one hour.

Studies have also explored the possibility of inducing fibrinolysis without thrombolytic agents. Thrombolytic drugs have not been employed and only a combination of micro-bubbles and ultrasound has been used. This has been shown to be a possible way of inducing fibrinolysis. A new *in-vitro* method has been developed for this purpose and *in-vitro* studies using micro-bubbles in combination with ultrasound has started [7]. The new mechanism found in our studies, where ultrasound

has been shown to affect biological tissue as well as chemical substances, has given us opportunity to develop the project further. Some of these experiments will be conducted in collaboration with Ass. Professor David Erlinge, Institutionen för Kliniska Vetenskaper, Lund; Sektion II–Kardiologi, Molekylär Kardiologi [12]. We aim to explore:

- the possibilities of using ultrasound in combination with microbubbles to induce thrombolysis.
- if the destructive effect on fibrin from acetylsalicylic medication can be increased by ultrasound exposure.
- if ultrasound exposure increase the release of ATP from red blood cells, with the effect of vascular dilatation, with intension for a treatment for ischemic tissue.

CO-WORKERS

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