1 Introduction

Some of the most common human cardiovascular diseases, such as myocardial infarction and stroke, share a common origin and are caused by thromboembolic events. In myocardial infarction, a coronary artery is occluded by a blood clot triggered by atherosclerotic plaque rupture. In stroke, ischaemia is caused by a thrombus that develops within the cerebral vasculature, or by an embolus from an atherothrombotic carotid artery or the fibrillating atrium.

Recent reports of stroke patient treatments using ultrasound signal a new therapeutic application of ultrasound. This tutorial introduces the principle of clot sonolysis, and discusses the physical ultrasound parameters involved. Ultrasound contrast agents enhance lysis. Clinical trials of the ultrasonic treatment of stroke are discussed.

2 Drug-induced Clot Dissolution without US

Strategies for dissolution of clots impeding or obstructing flow in a blood vessel, with the aim of reopening and re-establishing perfusion, have been pursued for several decades. A number of drugs have been shown to destroy the fibrin mesh in a thrombus (these are known as thrombolytic agents); the main thrombolytic agents currently in use are streptokinase, anistreplase, urokinase and the recombinant tissue plasminogen activators alteplase and reteplase (van Domburg RT et al. 2000**).** Thrombus dissolution using lytic agents has been successful in the clinical treatment of myocardial infarction and has become the standard clinical therapy world-wide. Major clinical trials have demonstrated the efficacy of the approach and a reduction in mortality. Direct intracoronary administration of streptokinase or urokinase has been shown to be superior to intravenous infusion (Simoons ML 1989). It should be noted, however, that percutaneous transluminal coronary angioplasty is preferred in many centres over intravenous thrombolytic therapy. A review of all major publications of the two treatment modalities revealed a better outcome for angioplasty (Keeley et al. 2003).

It took a while for the knowledge gained from clot dissolution in the heart to be applied in the brain, but the results obtained in this organ parallel the advances made in myocardial infarction. Clinical dissolution of thrombus was successfully achieved in large scale trials of the National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group (USA) (1995) and in Europe (Hacke et al. 1995), and intravenous recombinant tissue plasminogen activator is now the major validated therapy for acute ischaemic stroke.

3 Thrombolysis with Ultrasound - *in vitro*

3.1 Initial experiments

In 1992 two groups of researchers reported ultrasound facilitated thrombolysis (Francis et al. 1992, Lauer et al. 1992). Freshly prepared human blood clots labelled with fibrinogen were exposed to 1 MHz ultrasound in the presence of tissue plasminogen activator. Clot lysis with drug and ultrasound was greater than that obtained with drug alone. A better result was obtained when the sound intensity was increased, the time of exposure was prolonged, and the concentration of plasminogen activator was raised. Ultrasound released the fibrin degradation product D-dimer from the clot (Kimura et al. 1994).

In order to mimic the clinical situation better, perfusion systems have been built in which fibrin clots block narrow tubes, creating flow resistance. Exposing the clot under hydrostatic pressure to 170 kHz ultrasound and streptokinase shortened the time to reperfusion more than did 1 MHz (Olsson et al. 1994). A similar result was obtained with urokinase in another system in which flow was measured directly (Harpaz et a. 1994). In all experiments, controls were exposed to thrombolytic agent without ultrasound, resulting in less lysis . An overview of different approaches and techniques can be found in Pfaffenberger et al. (2005).

Clot dissolution by ultrasound has been verified many times since this. A number of parameters have been investigated.

3.2 Type of Thrombolytic Agent

Streptokinase and urokinase have both been found to be as potent as recombinant tissue plasminogen activator (Blinc et al. 1993). In

addition aspirin and heparin, first line drugs administered immediately to a patient with acute coronary infarct, were found to enhance thrombolysis synergistically when clots were also treated with low frequency ultrasound (Atar et al. 2003).

3.3 Ultrasound exposure Without drug

Two studies have investigated the effect of sound parameters on clot lysis in the absence of any fibrinolytic drug, investigating the loss of clot weight under different sound exposure conditions. In one study, clot dissolution was frequency and intensity dependent, with better dissolution being found at 20 kHz ultrasound than at 60 kHz, and with 0.2 W/cm² intensity being more effective than 0.12 W/cm² (Nedelmann et al. 2005). Thrombolytic efficiency in the second study depended directly on the pulse duration, its intensity, duty cycle and pulse length (Schaefer et al. 2005). Both studies confirmed that ultrasound can lyse a clot, even in the absence of a lytic drug.

3.4 Frequency Dependence

Of the list of acoustic parameters which may determine thrombolysis, frequency has been studied most often. The initial experiments employed ultrasound in the MHz range which tends to cause a temperature rise. 3.4 MHz led to less thrombolysis than lower frequencies (Blinc et al. 1993). In some further studies, low frequencies, which lead to less tissue heating, were used. Thrombolysis with 40 kHz ultrasound seemed to work effectively (Suchkova et al 1998), and a comparison between 100 kHz and 27 kHz showed the latter to be more effective (Suchkova et al. 2002). Low frequency was also used for investigations of the action of aspirin and heparin (Atar et al. 2003). All studies performed so far have favoured the use of a low frequency (in the sub-MHz range), including those mentioned above for which no thrombolytic agent was employed.

3.5 Sound Intensity and Duty Cycle

Sound intensity was varied from 1 to 8 Wcm^{-2} in the first experiments on thrombolysis, and temperature elevations of several °C were recorded. Lower intensities at low frequencies, many below 1 Wcm^{-2} have also been studied. A clear intensity dependence was seen in the range 0.25 Wcm^{-2} to 1.5 Wcm^{-2} (Suchkova et al. 1998). The initial lytic rate of clot lysis increased linearly with the duty cycle (Meunier et al. 2007).

3.6 Exposure Time

Initial insonation times to achieve thrombolysis were long, taking one to three hours to produce a clear effect. While later experiments have reduced this time to several tens of minutes, this is long compared with the time needed to reopen a thrombosed artery using transluminal percutaneous coronary angioplasty, which is only minutes. Furthermore these exposure times are longer than that required to infuse lytic drugs in both myocardial infarction and stroke. Exposure time therefore needs to be reduced for future application.

3.7 Mechanism of Clot Lysis

It is a general finding that low sound frequencies are most efficient at enhancing clot lysis. Several mechanisms have been proposed to explain the action of ultrasound. An early finding suggested that exposure of fibrin gels to ultrasound increased the pressure-mediated permeation of reactants into the clot. Lysis was considered to be promoted by cavitation-induced changes in fibrin gel structure (Siddiqi et al. 1995). An experiment on thrombus dissolution with plasminogen activator and 40 kHz ultrasound indicated that the supply of the drug to the clot surface was an important factor (Pieters et al. 2004). The finding that heparin and aspirin dissolved clots effectively with 27 kHz ultrasound at exposure times of 10 or 20 minutes lends further support for the action of cavitation (Atar et al. 2003). When ultrasound contrast agents (see below) were added to thrombi and subharmonic emissions were monitored, stable cavitation rather than inertial cavitation was found to be involved (Prokop et al. 2007).

3.8 Clot Lysis with Contrast Agents

Ultrasonic contrast agents are hard shelled gas-filled microbubbles with mean diameters of 3-5 µm size, small enough to pass through capillaries without getting stuck. When a

microbubble passes through an ultrasound field it is generally destroyed, depending on the pressure amplitude. In a first report of contrast agent enhanced ultrasonic thrombolysis, while reasonable levels of thrombolysis were seen with 170 kHz ultrasound and urokinase, significantly more was found when albumin coated microbubbles were added (Tachibana and Tachibana 1995). In a similar approach with galactose-based microbubbles and urokinase, clot lysis was 30% when a triple treatment with contrast, rt-PA and ultrasound was performed, about three times that for ultrasound and lytic agent alone (Cintas et al. 2004).

3.9 Clot Lysis in Animal Experiments

There is currently no animal model available which can mimic natural thrombotic or embolic vessel occlusion in the heart or brain. In the first published animal experiment on ultrasound mediated thrombolysis, jugular vein thrombosis was induced in rabbits by surgical vessel ligation. However, ultrasound could not achieve a significant increase in lysis (Lauer et al. 1992). Animal experiments showed lysis using tissue plasminogen activetor when clots were injected directly into the middle cerebral artery (Busch et al. 1997).

The temperature elevation in rat brain was was reported as 0.9°C for 340 kHz 1-7 Wcm^{-2} exposure for 30 minutes (Fatar et al. 2006). Low frequencies enhanced clot lysis, however it has recently been shown that the brains of rats exposed to 20 kHz ultrasound showed significant neuronal loss with circumscript cortical parenchymal necrosis (Schneider et al. 2006).

A stenosis which reduced flow in the femoral artery of rabbits by a half was used to generate a thrombus by injecting thrombin (Riggs et al. 1997). Ultrasound treatment with streptokinase dissolved the thrombus signifycantly more often than did infusing the enzyme, without ultrasound. Coronary artery occlusion was mechanically generated in pig coronaries using a catheter (Porter et al. 2001). When lysis was attempted with 40 kHz and 1 MHz ultrasound together with contrast agent, the thrombosed vessel was only successfully recanalised in a few cases.

4 Clinical Studies of Stroke

4.1 Without Contrast Agent

A small pilot study has demonstrated the feasibility of using ultrasound to recanalise an occluded middle cerebral artery (Cintas et al. 2002). In contrast to all subsequent studies and to current treatment guidelines, no thrombolytic drug was administered to the six patients involved.

Clot dissolution by ultrasound has now been studied in several clinical trials of acute stroke. In the largest study so far (290 patients), stroke from occlusion of the middle cerebral artery was treated in the standard way by administering tissue plasminogen activator and exposing for 120 minutes with 2 MHz transcranial Doppler ultrasound (Alexandrov et al. 2004). Unfortunately, no data on scanner output were provided. Clinical recovery occurred significantly more often after ultrasound treatment than for controls treated without ultrasound. In another trial, patients with cerebral vascular occlusion were treated with tissue plasminogen activator and exposed to 90 minutes of 300 kHz transcranial ultrasound at an intensity of 700 $mWcm^{-2}$ (spatial peak temporal average (Ispta)) from four transducers (Daffershofer et al. 2005). The trial was stopped prematurely since the ultrasound treatment caused signs of intracranial bleeding in the majority of patients. In addition, blood-brain barrier disruption was demonstrated in a patient treated with this device (Reinhard et al. 2006). A recent report suggested changing the transducer design to dual mode (i.e. power M-mode Doppler and low frequency ultrasound-mediated tPA thrombolysis) as this might cause less bleeding (Wang et al. 2008). The effectiveness of transcranial Doppler treatment of patients with middle cerebral artery occlusion has recently been confirmed (Eggers et al. 2008).

4.2 With Contrast Agent

Knowledge about enhanced clot lysis using ultrasound contrast agent has been transferred to stroke treatment, and clinical trials have been conducted. In a large study of 111 subjects with middle cerebral artery occlusion, patients were randomised to receive either microbubbles with ultrasound, ultrasound

without microbubbles, or standard thrombolytic therapy without ultrasound (Molina et al. 2006). Two hours after treatment started the occluded vessel was significantly more re-opened when microbubbles had been administered. It has also been shown that this treatment option is effective in stroke when the basilar artery has been occluded (Pagola et al. 2007). This enhanced effect of ultrasound contrast agents has been supported by another, smaller, study (Alexandrov et al. 2008). There was no difference between the galactose-based air-filled and sulphur hexafluoride-filled microbubbles used when compared clinically (Rubiera et al. 2008).

4.3 Clinical Studies: Myocardial Infarction

Surprisingly, there are no firm data about thrombolysis induced by ultrasound in myocardial infarction. The only evidence is in a short note about the experimental treatment of 25 patients which was published in 2003 (Cohen et al. 2003). 27 kHz ultrasound was administered transcutaneously concomitantly with standard thrombolytic therapy for 60 minutes to 25 patients with ST-segment elevation acute myocardial infarction. No information about the sound field or transducer output intensity were provided. No conclusion was drawn about the effectiveness of the therapy nor its adverse effects.

4.4 Safety Implications

Most of the experimental results suggest that ultrasound parameters different from the standard diagnostic equipment may lead to improved treatment efficacy. The lower frequencies used seem to show a more pronounced treatment effect. However, there is also evidence, that incautious choice of ultrasound different from the diagnostic parameter settings may cause severe side effects on brain tissue (Nedelmann et al 2008).

The TRUMBI clinical multicenter study on transcranial therapeutic application of 300 kHz ultrasound demonstrated an increased rate of cerebral hemorrhage and had to be stopped prematurely due to massive side effects (Daffertshofer et al. 2005). Ultrasound induced blood brain barrier disruption was suggested as one possible cause (Reinhard et al. 2006). In this study atypical intracranial hemorrhage had occurred at sites distant from ischemia, with subarachnoid and intraventricular hemorrhage, and parenchymal hemorrhage into nonischemic cerebral tissue.

Higher frequencies such as 2 MHz used in the CLOTBUST study, did not show this serious adverse effects but reasons for this difference are still unclear.

An in-vitro animal study on rats reported vasogenic and cytotoxic edema formation and even necrosis in healthy rat brain tissue using transcranial continuous wave 20 kHz ultrasound (insonation time 20 min, Schneider et al. 2006). These effects were dose dependent and were found at intensities ranging from 0.5 to 2.6 W/cm2. Intensities below this threshold caused no pathological findings on magnetic resonance imaging scans and histology specimens. Application of low intensity 20 kHz ultrasound (0.2 W/cm2) that had previously not shown side effects in healthy rat brain resulted in an increased death rate of animals subjected to embolic middle cerebral artery occlusion (MCAO, Wilhelm-Schwenkmezger et al. 2007). As histological evaluation had revealed excessive hemispheric infarction in some of the deceased animals, a potential adverse effect of ultrasound on the ischemic tissue had been postulated (Nedelmann et al. 2008).

One main outcome of all studies is that the balance between thrombolytic efficacy and harmful effects such as tissue heating depends mainly on the frequency used. This is important because high brain temperature seems to worsen cerebral infarction (Morikawa et al. 1992). Other changes caused by tissue heating can be blood perfusion increase, cell activation or influencing molecular effects such as protein synthesis or membrane integrity depending on the circulating blood and cerebrospinal fluid (Fatar et al. 2006).

However, exposure studies with mid-kilohertz ultrasound are needed, in order to establish output parameters in a coherent way (ter Haar et al 2011) that allows the comparison and calculation of the risk potential of possible bio-effects within different studies. Additionally intracerebral monitoring of the brain tissue temperature is advised whenever possible during these studies.

4.5 Summary and Prospects

Myocardial infarction and stroke are caused by thrombotic or embolic vessel occlusion. Thrombolytic therapy is the treatment of choice. Ultrasound enhances thrombus dissolution, and its action is further enhanced when ultrasound contrast agent is added to the thrombolytic agent. The role of physical ultrasound parameters in clot lysis is partially known, low sound frequencies dissolve clots fastest. Clinical trials in which stroke patients were treated with the standard thrombolytic therapy showed a better outcome when ultrasound was added. Better outcomes were also found in clinical studies when ultrasound exposures were carried out in the presence of contrast agents.

It is still not known with certainty whether ultrasound enhances clot dissolution in myocardial infarction.

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