

Review Article

Heparin and angiogenic therapy

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Introduction

Collateral circulation is an alternative source of blood supply to the myocardium if this is jeopardized by failure of the original vessel to provide adequate flow. Gradual occlusion, or a high grade stenosis of the coronary arteries, is frequently associated with collateral development in patients with coronary artery disease^[1]. Although the existence of collateral circulation in such patients is associated with improved clinical outcomes^[2], the net effect is inadequate to compensate for the flow deficit in the native coronary arteries. Why the flow reserve through collateral circulation is limited to half of that of normal coronary circulation is not clear^[3]. However, several pieces of evidence indicate that it is possible to promote collateral development intentionally to alleviate myocardial ischaemia under stressed conditions^[4,5].

Angiogenic therapy is defined as the intervention used to treat or prevent pathological clinical situations characterized by local hypovascularity by stimulating or inducing collateral development^[6]. Until our clinical study^[7], there had been no reports, except for an intense year-long exercise training programme^[4,5,8,9], showing enhanced collateral development through some kind of intervention. We have demonstrated for the first time that the combination of heparin and exercise-induced myocardial ischaemia enhances collateral function and growth in patients with stable effort angina^[7]. Recently, angiogenic growth factors such as basic fibroblast growth factor and vascular endothelial growth factor were successfully used to stimulate cardiac angiogenesis to improve collateral circulation not only in various animal models^[3,10,11] but also in clinical settings^[12,13].

Heparin was discovered by McLean in 1916. Since then, many investigators have contributed to elucidate the basic and clinical properties of heparin^[14]. It has recently been recognized that heparin potentiates the activity of growth factors^[15–19]. This property of heparin

has been applied to enhance the growth of collateral vessels in animal models^[20–22] and humans^[7,23–25].

Heparin in angiogenesis

In 1982, Taylor and Folkman^[15] showed that heparin promoted angiogenesis of the chorioallantoic membrane of the chick embryo, which was induced by tumour extracts from human hepatoma cells. The important role of heparin in angiogenesis was suspected due to the following observations. A large number of mast cells containing heparin gather around rapidly growing capillaries in the vascularized areas of tumours^[26]. The increased endothelial migration occurs at a heparin concentration of $10 \mu\text{g} \cdot \text{ml}^{-1}$, although other mast cell products could not potentiate endothelial migration^[27].

Recently, numerous angiogenic growth factors have been purified and their amino acid sequences determined with subsequent gene clonings^[19]. Purification of these growth factors was facilitated with the use of a heparin column, since most angiogenic growth factors bind to heparin with high affinity^[16,28].

More recently, the mechanisms of heparin angiogenic potentiation have been widely investigated. Fibroblast growth factor interacts with heparin at multiple points after being released into the extracellular matrix until it activates intracellular signalling events in the responding cell. Fibroblast growth factor binds to pre-existent heparin in the extracellular matrix. Thus, heparin tethers growth factor to the extracellular matrix and creates a reservoir of the factor in a thermo-stable and protease-resistant form^[18,29,30]. In the case of fibroblast growth factor-mediated signal transduction, the growth factor is cross-linked as dimers and oligomers in the presence of heparin, suggesting that heparin-mediated fibroblast growth factor dimerization or oligomerization may be important for its receptor activation^[31–33].

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Animal studies

In 1981, Franklin *et al.*^[34] first showed that repeated 2 min coronary artery occlusions can induce collateral

vessels adequate for resting metabolic requirements in the area perfused by the occluded coronary artery in dogs. In this model, the total occlusion time needed for adequate collateral development was a quantitative index of the stimuli for collateral growth^[35]. Using the repeated coronary occlusion model, we investigated the effect of heparin treatment on collateral development^[20]. In eight control dogs, 2 min coronary occlusions were repeated 129 ± 44 times until acute occlusion was no longer accompanied by a reduction in systolic shortening of the ischaemic area. In a further eight dogs pre-treated with heparin, however, only 81 ± 33 occlusions were necessary to produce the same results^[20].

Previously Unger *et al.*^[21] evaluated the usefulness of heparin administration in their newly developed canine model^[36]. After placement of an ameroid constrictor on the left anterior descending coronary artery, and implantation of a left internal mammary artery into the ischaemic myocardium, heparin was infused into the ischaemic area. It was observed that selective infusion of heparin into the internal mammary artery promoted the formation of anastomoses between this artery and coronary vessels more effectively compared to placebo treatment^[21].

The efficacy of heparin treatment on collateral growth was also documented in a pig model of myocardial ischaemia. Carroll *et al.*^[22] demonstrated that heparin has a positive effect on the restoration of collateral blood flow in mini pigs with ameroid constrictors attached to the left circumflex coronary artery.

All these findings indicate that heparin has the potential to accelerate and potentiate collateral development *in vivo*.

Human studies

In 1988, we extended the results of the previously mentioned canine study^[20] to patients with stable effort angina. Ten patients with angiographically proven coronary artery disease and effort angina were exercised 10 to 20 min following pre-treatment with a single intravenous dose of heparin (5000 units) twice a day for 10 days. The rate–pressure product at the onset of 0.1 mV ST depression and anginal pain increased by 19% and 35%, respectively. The angiographic collateral score also increased in these patients. In contrast, none of these indexes changed significantly in patients with exercise alone^[7].

The efficacy of heparin treatment combined with vigorous exercise was confirmed in a subsequent randomized study^[24]. In the study, 52 patients with effort angina were randomly assigned to receive one of two intravenous doses of low molecular weight heparin, enoxaparin (40 mg or 60 mg) or a placebo. Each patient underwent 20 treadmill exercise sessions 10 to 20 min following pre-treatment with enoxaparin or a placebo. Only in patients who received 60 mg enoxaparin pre-treatment, did the rate–pressure product at the onset of

angina increase significantly, by 16%. Furthermore, the extent of angiographically demonstrable collateral circulation to the area perfused by the completely obstructed coronary artery increased in 47% of the enoxaparin-treated patients, but only in 25% of the placebo patients. These findings are compatible with a hypothesis that a combination of exercise and enoxaparin induces collateral growth to the jeopardized myocardium.

The beneficial effect of combining heparin treatment with exercise stress on measured indexes of myocardial ischaemia was also reported by recent studies^[23,25]. Quyyumi *et al.*^[23] demonstrated, for the first time that exercise and low molecular weight heparin treatment lessen myocardial ischaemia, probably due to enhanced collateral function and growth. In the study, 23 patients with stable coronary artery disease were randomized to receive subcutaneous low molecular weight heparin, dalteparin (10 000 units, once daily) or a placebo. The patients were exercised to ischaemia three times a day for 2 weeks. The rate–pressure product at the onset of 0.1 mV ST depression increased in 80% of heparin-treated patients compared with 31% of placebo-treated patients. Gagliardi *et al.*^[25] applied the heparin treatment to patients undergoing cardiac rehabilitation. They randomized 36 patients with stable coronary artery disease and evidence of exercise-induced myocardial ischaemia into three groups: a control group (n=11) which received the conventional medical treatment; a second group (n=11) which underwent three cardiovascular exercise rehabilitation sessions per week over 12 weeks; and a third group (n=14) which had the same exercise programme and received 12 500 units of calcium heparin subcutaneously 20 to 30 min before each exercise session. Although the rate–pressure product at 0.1 mV ST depression was increased by 16% in the third group of patients, it remained unchanged in the other two groups.

It is tempting to speculate on the underlying mechanism of the heparin exercise treatment. Despite intense research on collateral circulation and angiogenesis, it is still unknown what initiates collateral growth following a high grade coronary stenosis. High grade coronary stenosis is accompanied by myocardial ischaemia^[37,38], a transcollateral pressure gradient associated with increased wall and shear stresses at the site of pre-existent collateral vessels^[39,40], and an increase in several angiogenic growth factors^[41,42], which are all important stimuli for collateral vessel growth. Intense exercise accelerates collateral development when the jeopardized area is perfused by a high grade stenosis^[4,5]. Exercise provides a vigorous stimulus for collateral development as a result of a provoked myocardial ischaemia and an increased shear stress due to an enhanced collateral blood flow. Heparin potentiates the activity of locally released growth factors^[15–19]. This effect causes a proliferation of endothelial cells and smooth muscle cells with subsequent generation of well-developed collateral vessels. Thus, heparin itself is not an angiogenic factor, but accelerates the development of collateral channels^[43]. The increased release of angiogenic growth

factors appears to be indispensable to the heparin treatment. This is the case in acute myocardial infarction. Indeed, the extent of collateral development during convalescence was shown to be more pronounced in patients treated with an intravenous infusion of heparin (170 to 220 units \cdot kg⁻¹) daily during the acute stage of infarction^[44].

Whether the beneficial effect of the heparin exercise treatment persists after discontinuation of treatment is an important point, since collateral vessel regression may hamper favourable clinical outcomes in patients undergoing this treatment. Although only limited data are available, it has been reported that the newly developed collateral vessels do not regress between 3 and 17 months after cessation of the treatment, when another exercise test is performed and indirectly assesses collateral function^[25,45].

There are some limitations to the clinical studies with combined heparin and exercise. First, each study consisted of a relatively small number of patients (10, 23, 36, 52 patients) and the beneficial effect of this treatment on exercise capacity was generally modest. Furthermore, some studies were not double-blind, placebo-controlled^[7,25]. Second, each clinical study used the increase in the rate-pressure product at the onset of 0.1 mV ST depression or angina as a primary end-point instead of collateral growth evaluated angiographically. Although collateral flow changes are successfully assessed by exercise myocardial perfusion scintigraphy at the same workload^[45,46], direct measurements of collateral blood flow velocity or distal pressure to the balloon-occluded coronary artery would provide more accurate information on collateral circulation^[47,48]. Thus, in future clinical trials these more sophisticated methods are needed for the evaluation of collateral functional changes.

Future directions

Collateral vessel growth in response to a high grade coronary stenosis consists of two different processes, i.e. arteriogenesis and angiogenesis. Arteriogenesis is defined as adaptational enlargement and growth of pre-existent intercoronary anastomotic channels, while angiogenesis is by definition the growth of new capillaries by sprouting^[49]. In human collateral vessel growth, arteriogenesis is much more important than angiogenesis because it is associated with remarkably increased conductance.

The maturation of native collateral vessels is thought to be triggered by the increased shear stress at the site of native collaterals as a result of a newly established pressure gradient across the collateral network. Subsequently, the increased expression of monocyte chemoattractant protein-1 is accompanied by monocyte invasion into the vessel wall^[50]. It is likely that the activated monocytes infiltrating the vessel wall produce growth factors that stimulate arteriogenesis.

In the clinical studies with combined heparin and exercise, it was postulated that augmented collateral blood flow as a result of intense and laborious exercise increases shear stress at the site of pre-existing collaterals. Therefore, heparin exercise treatment needs ECG monitoring during each exercise period and patient's co-operation with the treatment.

The above-mentioned favourable situation for collateral development is also provided by the application of enhanced external counterpulsation to patients with high grade coronary stenosis^[51]. The device provokes diastolic augmentation of the aortic pressure, thereby increasing not only the coronary blood flow, but also the collateral blood flow^[52]. The increased collateral flow results in the increased shear stress at the site of pre-existent collateral vessels. Indeed, in a well-controlled, randomized, multicentre clinical trial, it has been shown that enhanced external counterpulsation can effectively and safely improve exercise tolerance, medication usage and subjective angina pectoris complaints, as a result of collateral development, in patients with chronic stable angina^[53]. From the aforementioned conceptual framework, it is conceivable that enhanced external counterpulsation combined with heparin pre-treatment has great potential in the treatment of angina patients at high risk for a revascularization procedure, or for whom revascularization is not technically possible.

In summary, several clinical studies may demonstrate the efficacy and technical feasibility of therapeutic angiogenesis with heparin exercise treatment, although the interpretation of each study should be cautious. Further studies examining which type of patient benefits most from the treatment, and comparing the clinical efficacy obtained by therapeutic angiogenesis with angiogenic growth factors, are needed. The clarification of the precise mechanisms of the interaction of angiogenic growth factors with heparin would provide a new therapeutic protocol attenuating the deleterious sequelae of coronary artery disease.

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