This is the published version of a paper published in *International Cardiovascular Forum Journal*.

Citation for the original published paper (version of record):

Hellman, U. (2014)
Hyaluronan, a beneficial glycosaminoglycan that may affect the phenotype of cardiac hypertrophy: a hypothesis.
http://dx.doi.org/10.17987/icfj.v1i5.62

Access to the published version may require subscription.

N.B. When citing this work, cite the original published paper.

Permanent link to this version:
http://urn.kb.se/resolve?urn=urn:nbn:se:umu:diva-107640
Hyaluronan, a beneficial glycosaminoglycan that may affect the phenotype of cardiac hypertrophy – a hypothesis

Urban Hellman

Cardiology, Heart Centre and department of Public Health and Clinical Medicine, Medicine, Umeå University, Umeå, Sweden.

Abstract

Myocardial hypertrophy contribute to ventricular diastolic dysfunction and can lead to heart failure, arrhythmia and even sudden death. It have been shown that during development of hypertrophy the concentration of the glycosaminoglycan hyaluronan increases. The increased concentration correlates to the increased gene expression of fetal and extracellular matrix genes that is associated with cardiac remodeling.

Moreover it has been shown that high molecular weight hyaluronan depolarize the membrane potential of cells. The increase of hyaluronan in cardiac hypertrophy could hypothetically affect the resting membrane potential in cardiomyocytes and thus affect the conduction through the heart.

Hypothesis. The role of hyaluronan as a molecule adapting the extracellular matrix when the heart is growing could potentially develop to be harmful to cardiomyocyte resting membrane potential and hence contribute to the risk of arrhythmia.

Key words: Hyaluronan, cardiac, hypertrophy, fetal gene program.

Myocardial hypertrophy

Pathological myocardial hypertrophy is induced by stress signals, e.g. long standing hypertension, valvular heart disease, myocardial infarction, hypertrophic and dilated cardiomyopathy. The developing hypertrophy may initially attempt to preserve contractile performance and normalize wall stress. However, the increase in cardiomyocyte size and cardiomyocyte disarray is accompanied by an increase in the number of developed fibroblasts, causing fibrosis and increased ventricular stiffness. This is followed by further hemodynamic overload and hypertrophy resulting in a detrimental cycle of cardiac enlargement and myocyte atrophy. This can contribute to segmental as well as global ventricular diastolic dysfunction and lead to heart failure, arrhythmia and even sudden death.

Induction of cardiac hypertrophy has been shown to cause a predominance of the expression of fetal genes. Such gene expression is characterised by the preference of carbohydrates over fatty acids as a substrate for energy provision, the expression of immediate early response genes, e.g. c-myc and c-fos, and the expression of atrial natriuretic peptide (ANP), transforming growth factor-β (TGF-β), as well as isoform switches of sarcomeric proteins e.g. myosin heavy chains and α-actins.

Hyaluronan

As early as 1894, Carl Thore Mörner isolated a “mucin” from the vitreous humour, presumably consisting of hyaluronan contaminated with proteins. Subsequently, in 1934, Karl Meyer and John Palmer isolated the previously undescribed polysaccharide from bovine vitreous humour. They named it hyaluronic acid (HA) from hyaloid (vitreous) and uronic acid. The name hyaluronan was introduced in 1986 but the abbreviation HA remains.

HA is an unbranched glycosaminoglycan, present in the extracellular matrix of all vertebrates. It is polydisperse at varying chain lengths, occupying a large hydrated volume, up to 1000 times greater than its own dry volume. The chemical structure of HA is repeating disaccharides of glucuronic acid and N-acetyl glucosamine with the number of repeated disaccharides reaching up to 105, thus representing a molecular mass of ~20,000 kDa.

HA is synthesized on the cytosolic side of the cell membrane and the growing chain is transported through the cell membrane into the extracellular space. In a man or woman of 70 kg of weight, there are approximately 15 grams of HA, of which almost 5 g is found in the skin. HA is also a major part of the vitreous humour of the human eye and synovial joint fluid.

Most tissues of the body contain HA in various forms, as a freely circulating molecule bound to HA-binding proteins ‘hyaladherines’, which are loosely associated with the tissue or anchored to the cell membrane via receptors. Differences in their tissue expression, cell localisation and regulation explain how the simple structure HA can display such a wide range of functional activities. A complex of HA bound to hyaladherines has different properties and functions, depending on size and concentration of HA and the hyaladherine.

HA function: HA has a variety of functions in the body. It participates in the structure of the cartilage, where it is bound to the proteoglycan aggregan and regulates the distribution and transportation of plasma proteins in the tissues. It also regulates various cell functions such as cell proliferation, recognition functions, cell locomotion, inflammation regulation, and cell protection. Furthermore, HA modulates tissue hydration and stabilizes the extracellular matrix.

During tissue development or damage, HA binds receptors and induces signal pathways with subsequent regulation of cell motility, invasion, and
proliferation. In addition, HA networks show a high resistance towards water flow, thus forming barriers in tissue, even though water can freely diffuse in the network. Movements of macromolecules are also hindered in a HA network, whereas low-molecular weight molecules can diffuse more easily. The ability of HA to co-regulate cell behaviour during embryonic development, healing processes, inflammation and tumour development makes HA essential for tissue growth. HA concentration, size and organization have been found to change when tissues and organs differentiate and cells divide and migrate in an extracellular matrix rich in HA. Furthermore, HA-receptor interactions mediate at least three important physiological processes; signal transduction, HA internalization and pericellular matrix assembly. Several HA cell receptors have been identified, where CD44 is the main receptor and has been shown to be involved in many biological functions, e.g. retention and endocytosis of HA, angiogenesis, tumour invasion and metastasis, adhesion and rolling of lymphocytes and also cell migration.

HA synthesis and degradation: HA synthesis is catalysed by the enzyme hyaluronan synthases (HAS), three different types with different properties have been isolated in vertebrates. It is degraded by a group of enzymes called hyaluronidases (HYALs) or by oxidation. Six HYAL-like sequences have been found in the human genome where four express functional proteins, with HYAL1 and HYAL2 being the most active in somatic tissue. It has been estimated that almost a third of all HA in the human body is degraded and synthesized each 24 hours. The balance between synthesis and degradation of HA can be disrupted in pathological conditions and fragmented HA constitutes a potential harm to the tissues. Large HA molecules, so called native HA have been shown to have opposite effect to fragmented HA. While high molecular weight HA has an anti-inflammatory and anti-angiogenic effect, the oligomeric HA exhibits inflammatory and angiogenic effect.

Hyaluronidase-2 (HYAL2) deficient mice represents one of many existing models for experimental left ventricular hypertrophy.

Table 1: Correlation between the expression levels of genes JunB, c-myc, c-fos, Egr1, HAS 1 and HAS 2.

<table>
<thead>
<tr>
<th></th>
<th>JunB</th>
<th>c-myc</th>
<th>c-fos</th>
<th>Egr1</th>
<th>HAS1</th>
<th>HAS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Junb</td>
<td>1</td>
<td>.924**</td>
<td>.940**</td>
<td>.868*</td>
<td>.728</td>
<td>.970**</td>
</tr>
<tr>
<td>Myc</td>
<td>.924**</td>
<td>1</td>
<td>.969**</td>
<td>.664</td>
<td>.598</td>
<td>.818*</td>
</tr>
<tr>
<td>Fos</td>
<td>.940**</td>
<td>.969**</td>
<td>1</td>
<td>.758</td>
<td>.718</td>
<td>.859*</td>
</tr>
<tr>
<td>Egr1</td>
<td>.868*</td>
<td>.664</td>
<td>.758</td>
<td>1</td>
<td>.925**</td>
<td>.944**</td>
</tr>
<tr>
<td>HAS1</td>
<td>.728</td>
<td>.598</td>
<td>.718</td>
<td>.925**</td>
<td>1</td>
<td>.798</td>
</tr>
<tr>
<td>HAS2</td>
<td>.970**</td>
<td>.818*</td>
<td>.859*</td>
<td>.944**</td>
<td>.798</td>
<td>1</td>
</tr>
</tbody>
</table>

Pearson Correlation, Sig. 2-tailed. ** P<0.01, *P<0.05. Hyaluronan synthase 1 and 2 correlates with immediate early genes JunB, c-myc, c-fos and Egr1.
Mice embryos without Hyaluronan synthase-2 expression (Has2-/-), synthesize no HA and display severe cardiac and vascular abnormalities, caused by impaired transformation of the cardiac endothelium to mesenchyme, and die during embryonic development27.

**Hyaluronan in the cardiac fetal gene program**

Our studies on the involvement of HA in the development of cardiac hypertrophy have showed an early and pronounced increase in the cardiac expression of the genes Has1, Has2 and the HA receptor CD44 in aorta ligated rats. This was accompanied by an increase in HA concentration in rat hearts28. The expression levels of Has1 and Has2 correlated strongly with the transcription factors, JunB, c-fos, c-myc and Egr1, indicating strong association with the early transcriptional changes in the aorta ligated animals (Table 1)29. JunB, c-fos, c-myc, and Egr1 are immediate early genes (IEG), activated in response to stimuli mediated via Angiotensin II and/or mechanical factors30-33. They are well known as early regulators of cell growth and to precede the expression of cardiac hypertrophy markers, e.g. ACTA1, ANP and also extracellular matrix genes.

Furthermore, the HA concentration correlates with the gene expression of extracellular matrix constituents (Figure 1) and also ANP and TGF-β29.

The correlation of HA synthesis to the expression of ANP and TGF-β2, which is regulated by IEG’s, opens the possibility that HA is also part of the fetal gene program activated in cardiac hypertrophy. This was supported by the observation that cultured cardiomyocytes secrete, through exocytosis, soluble molecule(s), a still unknown factor, which after transfer to fibroblasts increase the synthesis of HA34. In addition, adding HA to the media of cultured cardiomyocytes showed that both oligomeric HA and native HA influenced transcription of cardiomyocyte genes. Thus, HA seems to influence the growing cardiomyocytes, which is in concordance with the observations of many other cell types needing increased HA synthesis for growth14-15. The early expression of HA synthases followed by an increase of its concentration correlating with the expression of genes associated with the fetal gene program and extracellular matrix genes, suggest that HA plays an important part in the development of cardiac hypertrophy.

**Hyaluronan and the cardiomyocyte resting potential**

A recent finding has shown that besides influencing cells through the interaction with cell membrane receptors, high-molecular weight HA also depolarizes the membrane potential in human fibroblasts, human embryonic kidney cells (HEK) and central nervous system neurons in a concentration-dependent manner by inducing an influx of K+ into the cells, while degradation of cell surface HA by hyaluronidases caused hyperpolarization35. The phenomenon can be explained on the basis that the negative charge of HA exerts electrostatic exclusion, which will affect the flow of positively charged ions combined with a steric exclusion of other molecules36, 37. If this effect is also true for cardiomyocytes it would potentially open interesting speculations. Changes in the cardiac action potential, irrespective of the background, can lead to significant rhythm disturbances in humans38. Many anti-arrhythmic drugs act on the cardiac action potential, e.g. beta blockers, which prolongs action potential duration through potassium channel blockade. Thus, beta blockers used commonly for the management of cardiac arrhythmias after myocardial infarction, treatment of hypertension and hypertrophic cardiomyopathy39-41 would counteract the influx of K+ ions, an increased concentration of HA could cause.

If the cardiomyocyte resting membrane potential is normal, all fast Na+ channels are closed and excitation will open them, causing a large influx of Na+ ions. If, however, the resting potential is less negative, some of the fast Na+ channels will be inactive and hence insensitive to opening, thus causing a lesser response to excitation of the cell membrane. For this reason, if the resting membrane potential becomes too positive, the cell may not be excitable and conduction through the heart may be delayed, hence an increasing risk of arrhythmias38.

Overall, HA exists in most parts of the cardiac tissue with a higher concentration around the muscle fibres than around individual cardiomyocytes (Figure 2). An increased presence of HA around single cardiomyocytes could theoretically affect their resting potential, with a subsequent delay of conduction and finally add to the risk of arrhythmia in cardiac hypertrophy already present due to fibrosis.

**Conclusion**

It has been suggested that HA is a key molecule in inflammation43. We hypothesize that HA may also be a key molecule in the development of cardiac hypertrophy, i.e. it is necessary for myocardial remodelling and changes in HA metabolism affect the cardiac tissue. Its amount increases with cardiac hypertrophy thus, providing an adaption of the environment designed for the extracellular structural proteins expressed by the fetal gene program. However, the increased concentration of HA could ultimately also be detrimental for cardiomyocyte resting membrane potential and hence contribute to the risk of arrhythmia.

More studies on HA in the heart are needed to further support the above hypothesis.
Correspondence to:
Urban Hellman PhD
Department of Public health and clinical medicine, Medicine
Umeå University
SE-901 85 Umeå, Sweden
E-mail: urban.hellman@umu.se

References


