The Intra- versus Extracellular Myocardial Tissue Sodium Contents in the Time Course of Infarct Healing

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Objective:

The purpose of this study was to measure the intra- and extracellular tissue sodium contents in myocardial infarction in the time course of infarct healing.

Background:

Tissue sodium content is elevated in myocardial infarction (MI).[1] Tissue sodium content has been studied experimentally and clinically in acute infarction and throughout infarct healing using ex vivo flame photometry and in vivo Sodium MRI (^{23}Na MRI):[2-6] In the acute phase of myocyte necrosis a progressive increase in total tissue sodium content has been observed. This increase is due to myocyte membrane rupture combined with sodium delivery into infarcted tissue. Tissue sodium content peaks in the first days of infarction. Later on, while infarct healing is on the way, a decrease in tissue sodium content has been measured. Finally, in scar tissue, tissue sodium content is less than in the first days of infarction, however, remains elevated when compared to remote, viable myocardium.

Recently, ^{23}Na MRI combined with a shift reagent (Thulium) has been used to the separate the intracellular and extracellular fraction of tissue sodium in ischemia and acute MI.[7,8] The present study was performed to measure the intracellular and extracellular tissue sodium contents in the time course of infarct healing.

Methods:

Experimental protocol: MI was induced in female Wistar rats by ligation of the left anterior descending coronary artery. Hearts were excised and perfused isovolumically in the Langendorff-mode either on days 1 or 3 post-MI or after 3 weeks. Krebs-Henseleit buffer contained 5 g/l of the Tm(DOTP)$^{5-}$ shift reagent to allow separation of the intra- and extracellular MR sodium signals. Time points of MRI were chosen such that sodium contents were measured in necrosis (day 1 post-MI), in granulation tissue (day 3) and in scar (3 weeks). After MRI hearts were cut in short axis slices to obtain Hematoxylin-Eosin and Picrosirius-Red Staining (HE and PSR, resp.) in order to confirm MI and track infarct healing.

MR imaging: MR experiments were performed on an 11.75 T Bruker AMX-500 microscopy system using a homebuilt birdcage resonator. A chemical shift imaging sequence (CSI, TR 38.8 ms, bandwidth 14 kHz, phase encoding time 420 ms) was applied to obtain 3D spatial and 1D spectral datasets with a nominal spatial resolution of 1x1x3 mm^3. CSI data sets with flip angles of 35º, 69º and 104° (center of resonator) were acquired as previously described,[9] in order to measure and correct for T_1 saturation. Intracellular and extracellular sodium images were reconstructed by integration of the intra- and extracellular resonance line in the spectral domain. A third 3D image set showing total tissue sodium content was obtained by addition of the intra- and extracellular ^{23}Na MR images.

MR image analysis: Remote myocardium was defined in the inferior or posterior wall, guided by anatomical landmarks (papillary muscle, right ventricle) and by HE staining, which excluded MI in this area. To delineate MI a signal intensity threshold of 2 standard deviations above signal intensity in remote myocardium in the MR images of total tissue sodium was used. Finally, sodium contents were quantified using signal intensity in buffer (known sodium content) as reference.

Results:

MI was induced in 112 rats. 67 animals survived and were submitted to MRI. After MRI 18 animals were excluded from analysis because HE staining did not confirm MI. The table shows the intra- and extracellular sodium contents in the remaining 49 animals (mean ± standard error, * denotes statistical significance of infarction versus remote as determined by paired, two-tailed Student's t-Test with Bonferroni correction for repeated comparisons, T = Tissue, MI = Myocardial Infarction).

Sodium Content (g/L wet weight)	Necrosis (n=14)		Granulation T. (n=18)		Scar (n=17)	
	MI	Remote	MI	Remote	MI	Remote
Intracellular	0,14 ± 0,03 *	0,33 ± 0,03	0,06 ± 0,02 *	0,32 ± 0,04	0,07 ± 0,02 *	0,3 ± 0,03
Extracellular	2,55 ± 0,09 *	1,69 ± 0,05	2,47 ± 0,09 *	1,69 ± 0,09	2,59 ± 0,12 *	1,66 ± 0,1

Discussion:

Intracellular sodium content was found to be low in acute MI and throughout all stages of infarct healing. As opposed, elevated levels of extracellular sodium were measured.

In acute MI (necrosis) these findings conform with the idea of membrane rupture and subsequent loss of cellular ion homeostasis. Similarly a loss of the intracellular potassium ion and an increase of the extracellular chloride ion have been measured in acute MI.[10,11] In the later stages of infarct healing the alterations of intra- and extracellular sodium content ressemble the alterations of the potassium and chloride ions as well,[2] however, the physiology to explain these alterations is less clear. To the best of our knowledge there are no studies available that have measured the extracellular and intracellular spaces in granulation tissue or in scar tissue post-infarction. The elevated extracellular tissue sodium content in granulation tissue and in scar suggests, however, that the extracellular space is large - when compared to remote - at these stages of infarct healing. These considerations would be in line with studies by Pereira et al., who have measured an increased volume of distribution for the extracellular MRI contrast agent Gd-DTPA in chronic MI.[12]

Conclusion:

Significant alterations of intracellular and extracellular sodium content were measured in necrosis, granulation tissue and scar tissue. This is the first study to measure the intracellular and extracellular fraction of sodium in acute MI and in the time course of infarct healing.

References:

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