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Regeneration processes in human myocardium after acute ischaemia – quantitative determination of DNA, cell number and collagen content

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Abstract We examined and compared 22 human hearts (6 normal hearts, 4 with hypertrophic cardiomyopathy and 12 after acute ischaemia) for their DNA and collagen content and their cell number. A positive correlation between total heart weight and DNA content was demonstrated in all hearts. The relative DNA content decreased and the rate of polyploidy increased in the infarcted and hypertrophied hearts. An aneuploid DNA content was found only in the infarcted hearts. Both hypertrophied and infarcted hearts showed a significantly higher collagen content than did the normal hearts. There was a positive correlation between collagen content and total heart weight in the hypertrophied hearts but not in the infarcted hearts. In the infarcted hearts, the increase in the relative collagen content in the left ventricle was significantly larger than that in the right ventricle. Heart cell number was higher than normal in some hearts with acute ischaemia, which was possibly a sign of compensatory regeneration of heart cells in hearts damaged by ischaemia.

Key words Collagen · DNA content · Cell number · Myocardial infarction · Polyploidization

Introduction

After acute ischaemia of the myocardium caused by hypoxia there is a decline in the number of heart muscle cells and therefore a considerable diminution of myocardial contractility. The heart is able to compensate for such damage to some extent, by means of hypertrophy of the remaining myocardium [11]. Hypertrophy also occurs under a high functional load, because of hypertension, aortic valvular stenosis or hypertrophic cardiomyopathy. All these morphological myocardial changes result in a histologically ascertained increase in connective tissue [1, 2, 15, 19].

Using quantitative DNA measurement and determination of the collagen content and of the total number of cells in 22 human hearts, we registered and interpreted a regeneration process in the myocardium.

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Materials and methods

We prepared 22 human hearts: 6 without any clinical or histopathological sign of heart disease, 4 with a clinical diagnosis of hypertrophic cardiomyopathy and 12 after acute ischaemia at various sites. After weighing (total heart weight), we prepared the hearts according to Müller [20], weighed them again (total myocardial weight) and separated them into six parts: anterior and posterior wall of the left and right ventricles, septum and outflow tract. We then took 3-cm^3 samples from the different sites (where there was a scar in the infarcted hearts we took samples of the surrounding tissue) and prepared them for light microscopy by common H&E and Van Gieson staining of 5-µm sections of tissue from the different sites, and for DNA determination using biochemical and cytophotometric methods. For biochemical determinations the specimens were homogenized according to Burton [10] and Dische [12] using an ultraturrax and centrifugation with perchloride acid, 0.25 M. Incubation and hydrolysis at 90°C using DNA solution followed (100 ml glacial acetic acid, 1.5 g diphenylamine, 1.5 ml sulfuric acid 96%, 0.1 ml acetaldehyde solution 2% [10]) for 17 h. Extinction was performed at 595 nm against a standard reference solution containing 10 mg DNA in 40 ml 0.5% perchloride acid (C. Roth, Karlsruhe).

For cytophotometry according to Böhm [4] homogenising was again performed using an ultraturrax and the material was spread out on poly-L-lysine (Sigma P-8920) slides. Hydrolyses for 100 min 4 N HCl at 28°C followed, with washing twice with distilled water for 60 min and staining with Schiff's solution (5 g pararosaniline in 150 ml 1 N HCl and 5 g potassium metabisulfite in 850 ml distilled water). After further washing with distilled water and SO_2 washing (6 g potassium metabisulfite in 60 ml distilled water, 60 ml 1 N HCl, distilled water at 1200 ml), dehydration was performed in a series of graded alcohol (70%, 96%, 100%). We measured a total of 400 nuclei per heart using a Vickers N86 scanning microdensitometer. As a reference standard, we used simultaneously stained haploid bull spermatozoa.

According to Stegemann [24], the collagen content can be determined by measuring the concentration of hydroxyproline. Neumann and Logan [21] correlated these variables quantitatively. The samples were dewashed in a series of graded alcohol, after which

Table 1 Total results of the quantitative measurements. The mean value of the measurements is shown in each case. (*m* male, *f* female)

Heart No, patient age and sex	Pathological findings	Total heart weight (g)	Total myocardial weight (g)	Total DNA content (mg)	DNA concentration (mg DNA/g myoc.)	Content of collagen (mg)	Number of heart muscle cells (x10^9)	Connective tissue cells (x10^9)
Normal hearts		326.5	168.18	50.28	0.315	1984.0	1.23	4.24
1; 54 years, m		250.0	119.24	49.58	0.416	1650.3	1.20	4.74
2; 54 years, f		288.0	100.74	41.40	0.411	1578.8	1.20	3.40
3; 56 years, m		337.3	165.79	50.17	0.303	2055.1	1.25	4.03
4; 62 years, m		344.0	197.38	50.80	0.257	2138.5	1.69	4.03
5; 59 years, f		355.0	211.70	53.21	0.251	2141.9	1.98	4.40
6; 72 years, m		385.0	214.60	56.50	0.253	2339.3	2.03	4.83
Hypertrophic hearts		435.3	215.17	41.32	0.216	9195.9	0.94	3.67
7; 71 years, f	Interstitial fibrosis	324.9	120.42	43.19	0.359	5079.5	1.07	4.14
8; 67 years, m	Apical aneurysm	402.0	215.08	38.13	0.177	8354.1	0.87	2.94
9; 72 years, m	Interstitial fibrosis	404.2	172.01	30.58	0.175	9651.5	0.93	2.28
10; 57 years, f	Interstitial fibrosis	610.0	353.15	53.36	0.151	13698.5	0.89	5.30
Infarcted hearts		410.4	204.34	51.85	0.237	9365.6	1.33	5.22
11; 58 years, f	Posterior wall	242.0	112.32	36.94	0.328	3962.8	0.73	3.34
12; 75 years, f	Posterior wall	278.5	153.55	26.39	0.172	10377.3	0.71	5.20
13; 53 years, m	Anterior wall	363.1	206.60	38.78	0.188	7632.1	1.33	6.70
14; 73 years, f	Anterior wall	371.9	198.84	36.32	0.183	12501.5	0.73	2.90
15; 67 years, m	Septum	383.4	269.01	67.04	0.249	7473.5	1.83	5.23
16; 72 years, m	Posterior wall	395.2	242.34	64.23	0.265	14758.4	1.95	8.35
17; 83 years, f	Posterior wall	421.5	160.11	43.09	0.269	3283.1	1.19	3.34
18; 79 years, m	Posterior wall	461.4	226.78	63.04	0.278	14258.5	1.50	6.66
19; 75 years, m	Septum	480.0	232.38	53.80	0.232	13262.6	1.26	4.48
20; 67 years, f	Posterior wall	480.9	216.40	51.95	0.233	9760.6	1.70	6.60
21; 69 years, m	Posterior wall	500.3	217.15	55.20	0.246	11346.8	2.05	6.15
22; 56 years, m	Anterior wall	546.3	216.64	45.45	0.204	3769.8	0.93	3.70

Table 2 Results of the qualitative determination of the ploidy classes and of the comparison of the relative content of collagen in left and right ventricles. The mean value is shown in each case.

	Relative content of collagen: right (% of myocardial weight)	Relative content of collagen: left (% of myocardial weight)	Ploidy classes (%)					Aneuploidy
			2c	4c	8c	16c	32c	
Normal hearts	1.72	1.65	18.89	50.78	25.44	4.50	0.39	0
1	1.6	1.3	24.66	54.00	20.66	0.66	–	–
2	1.9	1.49	16.66	45.66	30.66	7.00	–	–
3	1.4	1.2	12.00	42.33	32.33	11.33	2.00	–
4	1.7	1.8	18.33	53.00	23.66	5.00	–	–
5	1.9	2.1	20.33	54.33	24.33	1.00	–	–
6	1.8	2.0	21.33	55.33	21.00	2.00	0.33	–
Hypertrophic hearts	2.5	3.4	8.75	43.16	37.75	8.66	4.08	0
7	4.2	4.2	10.66	56.66	29.00	2.33	1.66	–
8	2.4	4.2	8.33	33.66	50.00	13.66	3.66	–
9	1.3	0.87	11.33	34.66	42.66	6.33	5.00	–
10	2.2	4.33	4.66	47.66	29.33	12.33	6.00	–
Infarcted hearts	2.8	5	8.5	43.86	32.97	10.50	1.97	2.67
11	4.16	3.3	12.00	33.33	37.66	8.00	4.33	4.66
12	1.5	7.77	5.33	60.66	26.00	6.00	2.00	5.66
13	2.5	4.1	8.33	46.33	45.33	–	–	–
14	1.55	7.69	18.00	25.00	27.66	15.00	9.00	3.00
15	1.7	3.08	10.33	36.00	35.00	9.33	–	9.33
16	5.5	6.2	7.33	59.66	28.66	4.33	–	–
17	0.92	2.26	3.66	47.33	43.33	4.00	1.66	–
18	3.6	6.6	14.33	45.66	29.00	10.00	1.00	–
19	1.8	6.7	10.00	36.33	34.33	10.00	–	9.33
20	1.9	5.3	9.00	46.33	5.00	38.33	4.33	–
21	5.3	5.2	2.66	52.00	39.30	6.00	–	–
22	1.72	1.94	1.00	37.66	44.33	15.00	1.33	–

we carried out hydrolysis with HCl 25% at 110°C and dilution using distilled water at a pH of 6. Sodium-acetate-citrate (120 g sodium-acetic acid, 50 g citrate acid, 34 g NaOH, 12 ml acetic acid, distilled water to give 1000 ml) was added plus chloramine-T solution (2.82 g chloramine-T, 40 ml distilled water, 60 ml methylene glycol, 100 ml sodium-acetate-citrate), diphenyl aminobenzaldehyde solution (5 g diphenyl aminobenzaldehyde, 50 ml methylene glycol monomethyl) and perchloric acid with mixing and incubation at 60°C. For extinction measurement we used a spectrophotometer and incorporated comparison with a standard solution (C. Roth, Karlsruhe).

After formalin fixation and paraffin embedding of the sample a 5-µm slice was subjected to vanGieson staining for determination of the cell number. Differentiated determination of 200 cell nuclei in an area of 10 μm^2 at a magnification of 200 and determination of the relation of nuclei of connective tissue cells to nuclei of heart muscle cells (CTC/HMC) were carried out, and the total cell number was calculated by using the total amount of DNA, the DNA ploidy classes, the CTC/HMC ratio and the total heart weight [1].

Results

From the results in Table 1 it can be seen that we found an increase of 25% in the total myocardial weight in the hypertrophied and in the infarcted hearts (infarcted hearts: 204.34 g; hypertrophied hearts: 215.17 g; normal hearts: 168.18 g). As a rule, the hearts did not exceed the critical heart weight of 500 g.

There was only a minimal change in the total DNA content of the damaged hearts compared with the normal hearts (infarcted hearts: 51.85 mg; hypertrophied hearts: 41.32 mg; normal hearts: 50.28 mg), but in general there seemed to be a weight-dependent increase in correlation with the total heart weight ($P < 0.1$; not significant). Similar results are found in the literature [25, 26].

The DNA concentration, like the relative DNA content, was significantly lower in the infarcted hearts (0.254 mgDNA/g myocardium) and the hypertrophied hearts (0.216 mg DNA/g myocardium), respectively, than in the normal hearts (0.315 mg DNA/g myocardium; $P < 0.05$). We also found a decrease in the DNA concentration with increased total heart weight ($r = -0.57$; $P < 0.01$).

The collagen content increased significantly, up to five times the normal value in the damaged hearts (normal hearts: 1984.4 mg; hypertrophied hearts: 9195.9 mg; infarcted hearts: 9365.6 mg; $P < 0.001$). There was a significant positive correlation between collagen content and total heart weight in the hypertrophied hearts ($r = 0.96$; $P < 0.05$), but not in the infarcted hearts.

There were only small differences in the calculated myocardial cell numbers between the damaged hearts (infarcted hearts: 1.33 thousand million; between hypertrophied hearts: 0.94 thousand million) and the normal hearts (1.23 thousand million). The number of connective tissue cells increased from 4.24 thousand million (normal hearts) to 5.22 thousand million (infarcted hearts). Overall, there was a positive correlation between the number of connective tissue cells and the total heart weight in the damaged hearts ($r = 0.46$; $P < 0.05$), corresponding to the result noted for collagen content.

Table 2 shows results on the relative content of collagen and the classes of ploidy. We determined a highly significant reduction of the proportion of cells with a DNA content of 2c (infarcted hearts: 8.5%; hypertrophied hearts: 8.75%; normal hearts: 18.89%; $P < 0.005$) as well as an increase in the 16c (infarcted hearts: 10.5%; hypertrophied hearts: 8.66%; normal hearts: 4.5%) and a significant increase in the 32c part (infarcted hearts: 1.97%; hypertrophied hearts: 4.08%; normal hearts: 0.39%; $P < 0.05$). Regarding the 4c part, we saw a tendency to decrease (infarcted hearts: 43.86%; hypertrophied hearts: 43.16%), while for the 8c part there was a tendency to increase (infarcted hearts: 32.97%; hypertrophied hearts: 37.75%) in the damaged hearts compared with normal hearts (4c: 50.78%; 8c: 25.44%). Heart cells with an aneuploid DNA content were found only in hearts with acute ischaemia (2.67%).

The relative collagen content (% of the total myocardial weight) of the right ventricle in comparison with the left ventricle showed a clearly greater increase in the collagen content of the left ventricle in the infarcted hearts (left: 5%; right: 2.8%). We also found a larger increase in the left ventricular collagen content in the hypertrophied hearts (left: 3.4%; right: 2.5%) than in the collagen content of normal hearts (left: 1.65%; right: 1.72%).

Discussion

Heart and circulatory disorders are the main cause of death in the Western world, and myocardial infarction and its consequences play an important part in this. Nevertheless, the clinical courses can vary widely in different patients who have suffered acute myocardial infarction. We wished to determine the processes of regeneration of the myocardium after myocardial infarction using biochemical, cytophotometrical and histomorphological methods.

We confirmed the complexity of morphological changes in the processes of regeneration. These processes are an attempt to compensate for the ischaemia-caused defect and for the loss of function of the damaged areas. The myocardium is able to repair the defect caused by the infarcted myocardium by increasing the collagen content. Although there is only a small increase in the number of connective tissue cells (4.24 thousand million in the normal hearts, 5.2 thousand million in the infarcted hearts), the collagen content of the infarcted hearts increases to fivefold that in normal hearts. This means that the connective tissue cells have to increase their synthesis of collagenous fibres considerably [1, 2, 15, 19]. On comparing the collagen content of the left and right ventricles separately, we saw that the increase in the collagen content was initiated mainly by the myocardial infarction. In this study, the acute ischaemic injury was always located in the left ventricle. In these infarcted hearts, the relative collagen content (% of total heart weight) of the left ventricle increased from 1.65% (in normal hearts) to 5%. Nevertheless, there was also an increase in the relative collagen content of the right ventricle, from 1.72% to 2.8%.

We also found an increase in the collagen content in the hypertrophied hearts. In these hearts, an oxygen diffusion disorder supervened, and the whole myocardium suffered from low-grade hypoxia [2]. The increased thickness of the myocardial wall caused by the hypertrophy results in an increase in the total collagen content to more than in the normal hearts and in a smaller difference of the relative collagen content in the right and the left ventricles compared with the infarcted hearts. The oxygen diffusion disorder often seems to become worse in a weight-dependent manner in parallel with the total heart weight [2, 16, 27], so that we found a correlation between the total heart weight and the content of collagen in the hypertrophied hearts.

The compensatory mechanisms invoked to maintain the myocardial function are complex. Myocardial cells have the ability to divide by mitosis only during fetal life and shortly after birth [14, 16]. Therefore, compensation for the loss of myocardial cells by an increase in cell number is not possible in older people. The heart can compensate in part for the loss of function after hypoxia-induced damage, by a number of mechanisms.

Heart muscle cells are normally able to become polyploid in the absence of increased strain, which is the reason why polyploid heart muscle nuclei occur in normal hearts [5, 7]. It is well known that amitotic division of the myocyte nuclei occurs as a result of increased workload [6], partly induced by sympatho-adrenergic stimulation [18], which leads to hypertrophy [11, 17, 23]. There is a so-called critical cell volume, which leads to doubling of the chromosomal content of the cell [3], allowing the synthesis of a greater amount of contractile proteins [22]. The growth of the cell is mainly lengthwise, because a constant diameter ensures better oxygenation [13]. We found decreased proportions of 2c and 4c cells and an increase in 8c, 16c and 32c cell components in all areas of the infarcted hearts, a sign of compensation attributable due to the increased strain. As a consequence of the oxygen diffusion characteristics mentioned above, like others [8, 9] we did not find cells with a DNA content more than 32c. Because of the decreased local oxygenation in the infarcted hearts we found fewer 32c cells around the infarcted areas than in the corresponding areas of the hypertrophied hearts, although the work demand is similar. Furthermore, we regard local occurrence of aneuploid cells as a sign of increased metabolism of the heart muscle, because they might be seen as an intermediate stage of chromosomal doubling (S-phase) [9]. In contrast to hypertrophy, myocardial infarction is an acute event leading to the need for a quick reaction of the heart muscle by way of polyploidization. In our study, aneuploidy as a sign of high chromosomal turnover was found only in the infarcted hearts.

Although the rate of polyploidization increased, the DNA concentration did not change: thus, there was an increase in the number of diploid connective tissue cells. Further, if there is an increase in the number cells and a

decrease in the DNA concentration, the contractility of the heart is adversely influenced [2]. In general, the DNA content seems to be constant in the infarcted hearts, while there is a numerical loss of myocardial cells and hypertrophy and polyploidization of the remaining cells.

It is interesting to note that in some infarcted hearts the number of heart muscle cells is greater than in the normal hearts, in our study and also in studies reported the literature [1, 14] . This may be a sign of numerical regeneration and, therefore, of an ability of the heart muscle cells to complete mitosis in adult life.

We have identified a complex series of regeneration processes in hearts after acute ischaemia, with an attempt by the heart muscle to compensate so as to produce sufficient ejection volume.

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