Virchows Arch [Pathol Anat] (1981) 390:205–210

Virchows Archiv A

Sarcomere Relaxation and Ischaemic Myocardial Injury

C.L. Berry[1], J. van der Walt[1], and R. Wyse[2]

[1] Department of Morbid Anatomy, The London Hospital, London E1, England
[2] Department of Paediatric Cardiology, Institute of Child Health, London, WC1, England

Summary. Material from hearts known to have had recent myocardial infarction and biopsies of dog hearts subjected to an experimental procedure producing ischaemic injury, were examined by polarising microscopy. A technique which depends on the relaxation of sarcomeres on ischaemic areas of myocardia was used and assessed for its value in the diagnosis of early myocardial infarction. We found no statistically significant difference in sarcomere lengths in ischaemic and control heart muscle in man. The dog study failed to show changes with a study period of up to 2 h after ligation. We do not support the suggestion that sarcomere length is a useful measurement in the demonstration of early myocardial ischaemic injury in man.

Key words: Myocardial ischaemia – Early infarction – Sarcomere relaxation

Introduction

An accurate diagnosis of myocardial infarction is often of considerable clinical and pathological importance. Methods used for clinical diagnosis include measurement of various serum enzymes (Hamoldky 1967; Nissen et al. 1965; Cohen 1964; Cohen et al. 1964), electro-cardiography and radioisotope cardiac imaging. Histopathologically the diagnosis of myocardial infarction is not difficult in patients surviving more than 18 h, but in those dying within this period there are many potential problems. Histochemical methods have been used to identify damaged muscle, that most frequently used being measurement of released mitochondral dehydrogenase (Nachlas and Schnitka 1963) but recent reviews suggest that none of these methods are effective for at least five hours after the onset of ischaemia (Anderson et al. 1979). Ischaemic change is accompanied by electrolyte and pH changes in the muscle, which have also been used by some workers to establish a definite diagnosis (McVie 1970; W.H.O. 1970).

Offprint requests to: Professor C.L. Berry, Department of Morbid Anatomy, The London Hospital, London E1, England

0340-1227/81/0390/0205/$01.20

Non-enzymatic methods which have been used include measurements of intracellular fat, phospholipid and PAS positive material resistant to diastase (Wartman et al. 1936; Niles and Barnhouse 1967). Changes in the uptake of a reactive protein and fibrinogen have been found and used as an index of ischaemia (Kushner et al. 1963; Kent 1967). Poley, Fobes and Hall (1961) have described a method of measuring the changes in affinity for acid and basic fuchsin, which has been employed in studies in hearts where minor or diffuse evidence of ischaemia might be exptected (Berry 1967; Berry et al. 1969). However, this method required careful standardisation to ensure reproducibility, and is not suitable for routine use.

Because of these difficulties we have assessed the technique of Shperling (1978) which depends on the relaxation of sarcomeres in ischaemic areas of the myocardium and which offers the prospect of early diagnosis of myocardial ischaemia.

Materials and Methods

Two studies were carried out, the first in man and the second in the dog.

In the human study, 10 patients who had suffered a cardiac arrest and died after a short period of time, varying from almost instantaneous to three hours after collapse, were compared with 10 age matched controls who had died from non-cardiovascular causes.

Interval times from onset of symptoms to death in the test human cases were, Cases 1–3, less than 45 min, Cases 4, 5, 1 h and 1 h 20 min respectively, Cases 6, 7, 8, approximately 1 h 30 min, Case 9, 2 h 45 min and Case 10, 3 h.

No electrocardiography (EGG) was performed on those patients dying before 45 min had ellapsed from the onset of symptoms. In Cases 5, 6, 8, 9 and 10 changes of myocardial infarction were seen, in Case 7 no ECG was performed.

In the ischaemic group, a recent occlusion of one of the coronary arteries was demonstrated at post-mortem and blocks of myocardium were taken at a right angle to the cardiac surface from the centre of the area of supply of the affected artery. In the control group, the coronary arteries were widely patent at post-mortem and similar blocks of myocardium were taken from matched areas of macroscopically normal heart. Because of essentially negative results, a dog study was then performed.

In the dog study, greyhounds of both sexes and weighing between 25 kg and 30 kg were anaesthetised with thiopentane sodium via a leg vein. After a cuffed endotracheal tube had been introduced, ventilation was maintained with a gas mixture of 2:1 oxygen and nitrous oxide using a manley pulmovent. Blood gases and pH were routinely monitored throughout the experiment using an I.L. 4.7 blood gas analyser, taking samples through a catheter introduced into the femoral artery. This line was also used to monitor arterial pressure. The electrocardiogram (lead II) was displayed on an oscilloscope throughout the experiment. The chest was entered via a left thoractomy and the left internal mammary artery was ligated to prevent later intercostal bleeding. Four ribs were severed, both close to the sternal border and 20–25 cm along their length. After intercostal dissection this window was retracted to expose the heart. The pericardium was fashioned into a cradle and two or three mid-myocardial branches of the left anterior descending artery were dissected out for about 5 mm near their origin. They were then ligated which produced an ischaemic zone of approximately 15 cm^2. Visible edges were sharp towards the base but more diffuse towards the apex. The heart was covered with a pad moistened with warm saline to maintain myocardial temperature. There were no episodes of ventricular fibrillation. Samples of myocardium to the full thickness of the wall straddling control, sharp border and ischaemic tissue were excised 25 min after ligation in 6 dogs and after 2 h in 2 dogs. Samples were washed clear of blood in warm saline and placed in 10% buffered formalin. After fixation blocks were taken from normal and ischaemic muscle.

Table 1. Human hearts – controls

Case No.	No. of sarcomeres examined	Mean length (MCM)	Range (MCM)	Distribution (percentage)			
				<1.3 MCM	1.3–1.64 MCM	1.65–2.0 MCM	>2.0 MCM
1	332	1.8825	1.56–2.27	0	16	32	52
2	408	1.5319	1.13–2.27	16	52	28	4
3	415	1.5060	1.19–1.91	28	36	36	0
4	384	1.6276	1.13–2.08	4	44	48	4
5	417	1.4988	1.25–1.78	16	52	32	0
6	352	1.7756	1.46–2.27	0	32	40	28
7	363	1.7218	1.38–2.08	0	20	72	8
8	394	1.5863	1.13–2.27	12	32	44	12
9	377	1.6578	1.13–2.08	12	20	64	4
10	441	1.4172	1.05–2.08	44	32	16	8
Total	10 hearts	1.6206 (±0.14)	1.05–2.27	13.2	33.6	41.2	12.0

Table 2. Human hearts – infarcts

Case No.	No. of sarcomeres examined	Mean length (MCM)	Range (MCM)	Distribution (percentage)			
				<1.3 MCM	1.3–1.64 MCM	1.65–2.0 MCM	>2.0 MCM
1	389	1.6067	1.19–1.92	12	28	60	0
2	426	1.4671	1.00–1.92	24	28	48	0
3	361	1.7313	1.38–2.27	0	28	64	8
4	413	1.5133	1.00–2.50	28	20	28	24
5	431	1.4501	1.13–2.08	36	32	28	4
6	366	1.7077	1.32–2.08	0	28	68	4
7	365	1.7123	1.13–2.08	4	20	64	12
8	381	1.6404	1.05–2.08	16	16	52	16
9	376	1.6622	1.13–2.08	16	8	68	8
10	357	1.7507	1.38–2.50	0	20	56	24
Total	10 hearts	1.624 (±0.11)	1.00–2.50	13.6	22.8	53.6	10.0

Table 3. Dog hearts – controls (25 min)

Dog	No. of sarcomeres examined	Mean length (MCM)	Range (MCM)	Distribution (percentage)			
				<1.3 MCM	1.3–1.64 MCM	1.65–2.0 MCM	>2.0 MCM
1	389	1.6067	1.46–1.78	0	60	40	0
2	379	1.6491	1.38–1.78	0	32	68	0
3	422	1.4810	1.00–1.67	8	80	12	0
4	450	1.3889	1.05–1.78	28	56	16	0
5	425	1.4706	1.05–1.78	20	56	24	0
6	394	1.5863	1.13–2.27	12	32	44	12
Total	6 hearts	1.530 (±0.10)	1.00–2.27	11.33	52.67	34	2

Table 4. Dogs – control (2 h)

Dog	No. of sarcomeres examined	Mean length (MCM)	Range (MCM)	Distribution (percentage)			
				<1.3 MCM	1.3–1.64 MCM	1.65–2.0 MCM	>2.0 MCM
1	374	1.671	1.56–1.92	0	36	64	0
2	381	1.64	1.46–1.78	0	44	56	0
Total	2 hearts	1.66 (±0.02)	1.46–1.92	0	40	60	0

Table 5. Dog hearts – infarcted (25 min)

Dog	No. of sarcomeres examined	Mean length (MCM)	Range (MCM)	Distribution (percentage)			
				<1.3 MCM	1.3–1.64 MCM	1.65–2.0 MCM	>2.0 MCM
1	420	1.4880	1.05–1.08	20	48	28	4
2	389	1.6067	1.25–2.08	8	36	52	4
3	389	1.6067	1.25–1.78	4	44	52	0
4	459	1.3617	1.00–1.78	24	60	16	0
5	389	1.6067	1.25–2.25	8	52	28	12
6	409	1.5281	1.19–1.92	24	36	40	0
Total	6 hearts	1.533 (±0.89)	1.00–2.25	14.67	46	36	3.33

Table 6. Dogs – infarcted hearts (2 h)

Dog	No. of sarcomeres examined	Mean length (MCM)	Range (MCM)	Distribution (percentage)			
				<1.3 MCM	1.3–1.64 MCM	1.65–2.0 MCM	>2.0 MCM
1	517	1.209	0.89–2.08	50	34	14	2
2	456	1.371	0.89–1.92	32	48	20	0
Total	2 hearts	1.285 (±0.11)	0.89–2.08	41	41	17	1

In both studies, the blocks of myocardium, both ischaemic and control, were routinely processed through paraffin and stained with haematoxylin and eosin. The sections were then examined under polarized light, using a standard light microscope. The areas of longitudinal muscle fiber orientation were divided into five equal zones excluding the subendocardial zone. Using a calibrated micrometer, at a magnification of 800×, five myofibres within each zone were selected at random and the number of sarcomeres between two points on the micrometer scale counted. The two points selected were exactly 25 μm apart and from this it was possible to calculate the average sarcomere length for that segment of myofibres.

To eliminate observer bias, the sections were chosen by one investigator and the microscopy done by another, who did not know to which group the specimens belonged.

Standard statistical methods were used, Students '*t*' test was the basis of comparisons between groups.

Results

The results are shown in the series of Tables 1–6. No statistically significant differences were found between any of the groups examined.

The rationale of this works depends on the suggestion that the presence of relaxed sarcomeres in the myocardium indicates loss of contractility. Viable muscle cells may contract after death (Hort 1965) but cells that have been anoxic for some time before their death are unable to do so. Jennings and Ganote (1975) have suggested that if more than 50% of the sarcomeres are relaxed, irreversible ischaemia has occurred.

Experimental data suggests that it is probable that ischaemic change becomes irreversible after about 20 min (Jennings et al. 1969).

We found no statistically significant difference in the sarcomere lengths in ischaemic and control heart muscle in man. Nor were significant changes found in the dog study which extended up to 2 h after ligation of small coronary vessels.

However, it is important to note that most breeds of dog have an extensive coronary collateral circulation and whilst we are confident that a reduction of blood flow to below 5–10% of normal occurred in the centre of the ischaemic zone in our animals, which would kill cells rapidly, there will be a peri-infarct zone in which viable cells will persist. Factor et al. (1978) showed that infarcts may be of very irregular shape and some extension of the range of sarcomere length in the dog results suggest that a mixture of dead and viable cells may have been measured.

Our failure to observe significant changes in man is difficult to explain on technical grounds.

We only examined cases with established coronary artery occlusion and where we were confident of the historical details in terms of the timing of the episode. The period elapsed should suffice for the demonstration of the changes and although Shperling (1978) only studied cases with an interval of more than 4.5 h after onset of symptoms, he was able to find changes more rapidly in his rat studies.

From this study we cannot support the suggestion that sarcomere length is a useful measurement in the demonstration of early myocardial ischaemic injury in man.

References

Anderson KR, Popple A, Parker DJ, Sayer R, Trickey RJ, Davies MJ (1979) An experimental assessment of macroscopic enzyme techniques for the autopsy demonstration of myocardial infarction. J Pathol 127:93

Berry CL (1967) Myocardial ischaemia in infancy and childhood. J Clin Pathol 20:38
Berry CL, Tawes RL, Aberdeen E, Graham G (1969) Myocardial ischaemia in infants: its role in three common congenital cardiac anomalies. Ann Thorac Surg 8:383
Cohen L (1964) Contributions of serum enzymes and isoenzymes to the diagnosis of myocardial infarction. Mod Concepts Cardiovasc Dis 36:43
Cohen L, Djordjevich J, Ormiste V (1964) Serum isoenzyme patterns in cardiovascular and other diseases, with particular reference to acute myocardial infarction. J Lab Clin Med 64:355
Factor SM, Sonnenblick EH, Kirk ES (1978) The histologic border zone of acute myocardial infarction – islands or peninsulas? Am J Pathol 92:111
Hamolsky MW (1967) Enzymes in acute myocardial infarction. Circulation 35:427
Hort W (1965) Ventrikeldilatation und Muskelfaserdehnung als früheste morphologische Befunde beim Herzinfarkt. Virchows Arch path Anat 339:72
Jennings RB, Ganote CE (1975) Structural changes in the myocardium during acute iscahemia. In: Chasov Y, Bramwald E (eds) Myocardial metabolism (Proceedings of the 1st Soviet-American Symposium, USA) Medicine, Moscow, p 325–341
Jennings RB, Sommers HM, Smyth GA, Flack HA, Linn H (1969) Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. AMA Arch Pathol 70:68
Kent SP (1967) Diffusion of plasma proteins into cells a manifestation of cell injury in human myocardial ischaemia. Am J Pathol 50:623
Kushner I, Rakita L, Kaplan MH (1963) Studies of acute phase proteins. Localisation of C reactive protein in heart in induced myocardial infarction. J Clin Invest 42:286
McVie JG (1970) Port-mortem detection of inapparent myocardial infarction. J Clin Pathol 23:203
Nachlas MM, Schnitka TK (1963) Macroscopic identification of early myocardial infarction by alterations in dehydrogenase activity. Am J Pathol 43:379
Niles NR, Barnhouse DL (1967) The acid haematin-stain and myocardial damage. Arch Pathol 83:407
Nissen NI, Ranlov P, Weis-fogh J (1965) Evaluation of four different serum enzymes in the diagnosis of acute myocardial infarction. Br Heart J 27:520
Poley RW, Fobes CS, Hall MJ (1961) Fuschsinophilia in early myocardial infarction. Arch Pathol 77:335
Shperling ID (1978) The relaxation of sarcomeres in ischaemic injury of myocardium. Virchows Arch A Path Anat and Histol 380:149
Wartman JB, Jennings RB, Yokoyana HD (1936) Fatty change of the myocardium in early experimental infarction. Arch Pathol 62:318
WHO Scientific Group (1970) The pathological diagnosis of acute ischaemic heart disease. WHO Tech Rep Series, 441:5

Accepted October 1, 1980