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Delay of development of transmural irreversible ischaemic injury in canine myocardium

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Summary. During the course of experimentally induced myocardial ischaemia affected tissue initially suffers reversible ischaemic injury or, if ischaemia persists, injury of increasing severity before becoming irreversibly damaged. This state is characterized by tissue necrosis and referred to as myocardial infarction. The purpose of this study was to investigate whether it is possible to delay or perhaps even prevent the development of irreversible ischaemic injury. Ischaemia was induced by coronary artery occlusion (CAO) in canine hearts for 90 min or 24 h. The drug used for intervention was hyaluronidase. Ischaemic damage was assessed by p-NBT staining and ultrastructural evaluation of tissue biopsies. Development of irreversible ischaemic damage was prevented during 90 min of CAO. However, progression of reversible to irreversible ischaemic injury could not be prevented during 24 h of CAO. In conclusion, it is possible to prevent the development of irreversible ischaemic injury by a suitable intervention during the early stages of ischaemia in the canine heart and thus to gain time for additional intervention in the early treatment of myocardial infarction.

Key words: Ischaemic injury – Myocardial ultrastructure – Hyaluronidase

Introduction

Occlusion of a coronary artery results in ischaemia of the myocardium perfused by this vessel. Ischaemia resutls in damage where the function and morphological appearance of this tissue may be impaired to a varying degree, depending on the duration of occlusion. The damage may be of reversible nature, implying complete structural and functional recovery after restoration of blood flow, or it may progress to irreversible injury resulting in cellular death and tissue necrosis. This is generally referred to as "myocardial infarction".

During the past years enormous effort has been made to limit the amount and degree of ischaemic damage following coronary artery occlusion under experimental and clinical conditions. One aspect studied has been the delay or avoidance of the progression of reversible to irreversible ischaemic injury in order to gain time for potential myocardial salvage.

In this study we investigated the possibility of delaying or avoiding progression of ischemic injury during short (90 min) and long term (24 h) experimental coronary artery occlusion in the canine heart, using ultrastructural analysis of tissue biopsies. The drug administered in this study was hyaluronidase which, although its mode of action is not yet completely understood, has been credited with reduction of infarct size under experimental conditions (Maroko et al. 1972; Braunwald and Maroko 1976a, b; Maclean et al. 1976; Kloner et al. 1978). It has also already been used in the treatment of myocardial infarction under clinical conditions (Oliveira et al. 1959; Braunwald and Maroko 1976a, b; Maroko et al. 1977).

Material and methods

Twenty-one mongrel dogs of either sex, unknown age, and a body-weight between 24 kg and 32 kg were evaluated in this study. All animals were premedicated with piritramide (5 mg/kg body weight). Anesthesia was initiated by injection of sodium pentobarbital (either 5 mg/kg or 10 mg/kg body weight, i.v.) and maintained with an $N_2O:O_2$ mixture of 2:1. Artificial respiration was performed with an Engström respirator. Heart rate, $M\dot{V}O_2$, coronary flow, cardiac output, and blood gasses were monitored during the entire length of the experiments.

The animals were divided into 3 experimental groups.

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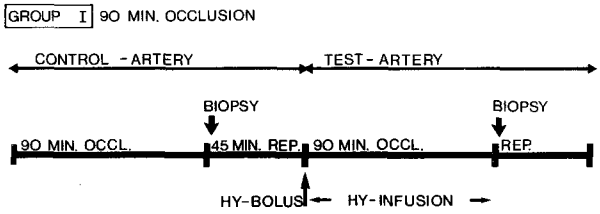


Fig. 1. Experimental protocol for animals belonging to group I (90 min occlusion). Consecutive occlusion and re-perfusion periods were performed on a control and on a test artery, as indicated above. The lower part shows the duration of occlusion (*OCCL*) and re-perfusion (*REP*) periods, the time of HY application, and the time-points at which tissue biopsies were taken

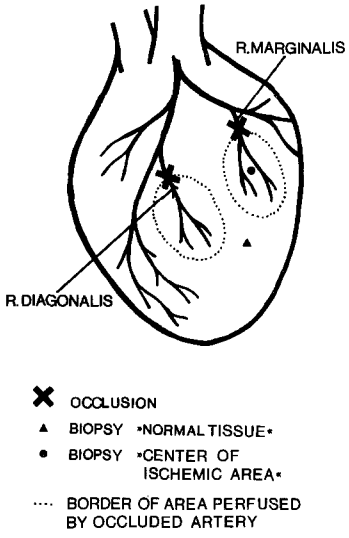


Fig. 2. Demonstration of occluded arteries and areas from which biopsies were taken for animals belonging to group I

Group I (8 animals). Figure 1 shows the experimental protocol for animals belonging to group I (90 min occlusion). Following thoracotomy via the 5th intercostal space a medium sized diagonal branch (R. diag.) of the left anterior descending coronary artery as well as a marginal branch (R. marg.) of the circumflex coronary artery were prepared.

Both arteries perfused areas of approximately the same size and were separated by tissue supplied by vessels other than those prepared as viewed by inspection. Following haemodynamic stabilisation, one of the prepared arteries was occluded by ligation for 90 min. Thereafter 30–45 min of reflow were allowed for haemodynamic stabilisation. Subsequently, hyaluronidase (HY) (Schering AG, Berlin) was injected as a bolus (500 IU/kg bodyweight, i.v.) and occlusion of the second coronary artery, again by ligation, followed.

During the following 90 min test occlusion HY was infused constantly (500 IU/kg bodyweight, i.v.). Reflow was also allowed after this occlusion period before the animal was sacrificed by an overdose of sodium pentobarbital. Either the R. diag. or the R. marg. was used as the test artery in animals in alternate sequence. The technique for producing 2 ischaemic areas of approximately equal size has been described by W. Schaper et al. (1979).

As shown in Fig. 2 biopsies for electron microscopy were taken from the left ventricle of the beating heart either immediately following control occlusion (4 animals) or test occlusion

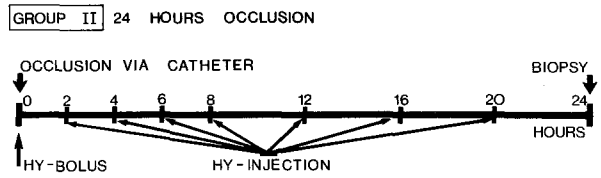


Fig. 3. Experimental protocol for animals belonging to group II (24 h occlusion). Application of HY was performed immediately following occlusion of the LAD via catheter and at 2 h, 4 h, 6 h, 8 h, 12 h, 16 h, and 20 h thereafter. Tissue biopsies were taken before termination of the experiments after 24 h of occlusion

(4 animals) with a Tru-cut needle (Travenol®). Biopsies evaluated in this study were taken from the following areas: 1. From the macroscopically visible center of the ischaemic area which was akinetic, bulged outward and/or was cyanotic, termed "infarct tissue" and; 2. from normally perfused tissue, termed "normal tissue".

Biopsies were approximately 8–15 mm in length and 0.8 mm in diameter. They were immediately divided into a subepi- and subendocardial section in a drop of cold (4° C) glutaraldehyde and further fixed by immersion in glutaraldehyde at 4° C.

At the end of the experiment hearts were removed and infarct size was measured by p-NBT staining (Schaper et al. 1979).

Group II (5 animals). Figure 3 shows the experimental protocol for animals belonging to group II. A modified Judkins catheter carrying a guttapercha cone on its tip (diameter in 0.1 mm × kg bodyweight) was placed transfemorally into the left main coronary artery, and a bolus of HY (500 IU/kg bodyweight) was injected. Thereafter the LAD was embolished by stripping off the guttapercha cone after proper positioning. During the following 24 h of CAO dogs were allowed to recover, and at 2 h, 4 h, 6 h, 8 h, 12 h, 16 h, and 24 h post occlusion HY (200 IU/kg bodyweight, i.v.) was injected. At the end of the occlusion period animals were reanesthetised, thoracotomy was performed, and biopsies were taken from the center of the ischaemic areas and normally perfused tissue as before. The animals were then sacrificed, hearts were excised, and infarct size was determined as previously described.

Group III (8 animals). The experimental procedure for this group was the same as in group II with the exception that no HY was administered. Biopsies from this group served as controls for those taken from group II.

Preparation for electron microscopy. All biopsies were immersion fixed for 3–10 h in cold (4° C), 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4, 400 mosm). They were then rinsed repeatedly in cacodylate buffer supplemented with 7.5% sucrose, postfixed in 1.6% osmiumtetroxide (OsO_4) in veronal acetate buffer, rinsed in veronal acetate buffer supplemented with 7.5% sucrose dehydrated in a graded ethanol series, treated with propylene oxide and embedded in Epon (LADD Industries, Burlington, Vermont, USA). Semi-thin sections 1–2 μm in thickness were prepared and stained with Toluidinblue. Following light microscopic examination of the tissue, for location of artefact-free areas, 50 nm thin sections were cut, mounted on uncovered copper grids, contrasted with uranyl acetate and lead citrate, and finally viewed in an EM 300 Philips electron microscope.

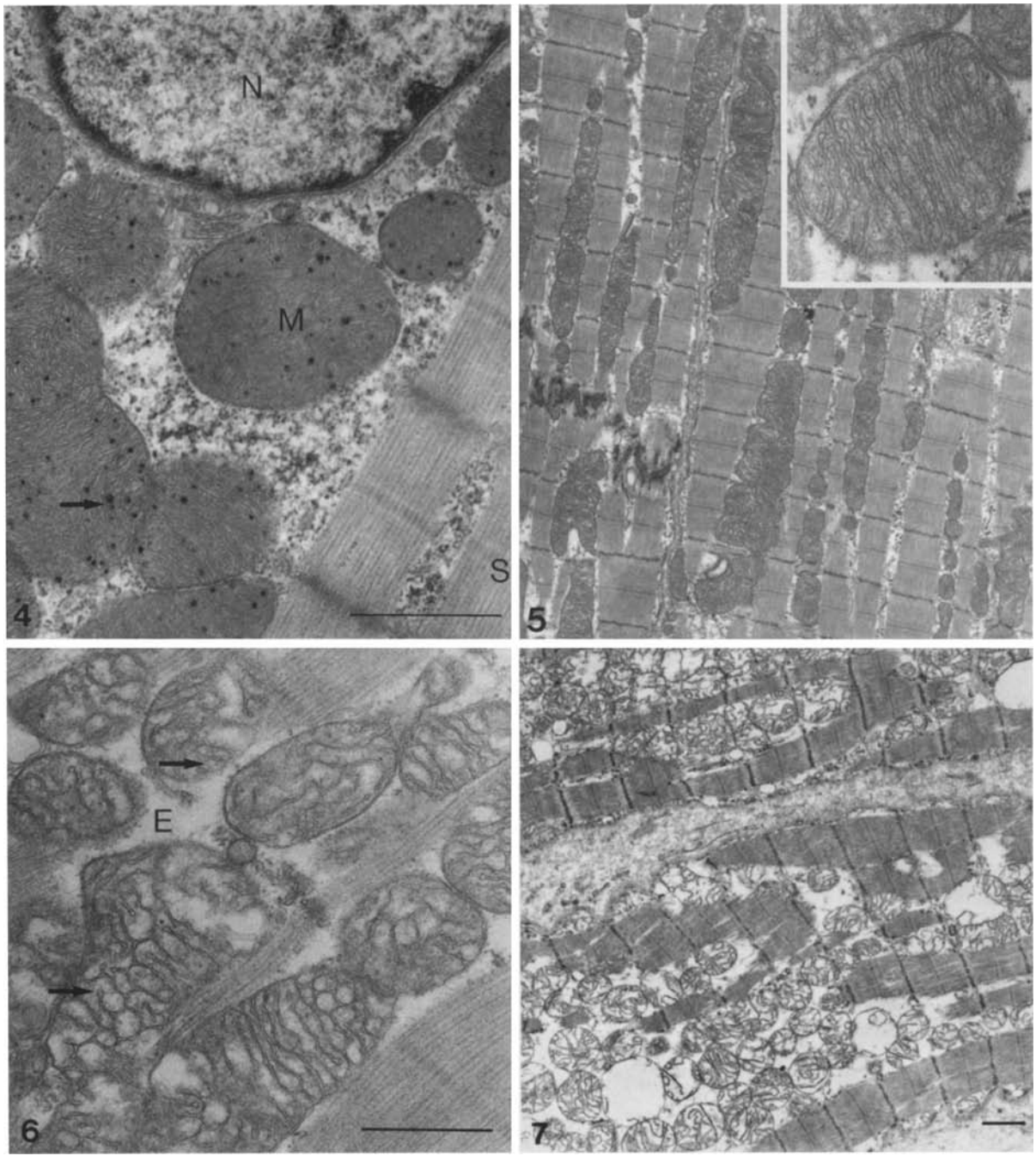


Fig. 4. Normal myocardium. Mitochondria (*M*) contain a dark, homogeneous matrix and numerous matrix granula (→) and are surrounded by slightly contracted sarcomeres (*S*) in register. Nuclear (*N*) chromatin is evenly and finely dispersed. Magn ×24000

Fig. 5. Slight ischaemic injury. Mitochondrial matrix granula are no longer present (*see inset*) which is characteristic for this degree of ischaemic injury. Sarcomeres (*S*) are in register. Part of the intercalated disc (*ID*) is visible. Magn ×6600, Inset ×39000

Fig. 6. Moderate ischaemic injury. Damage to mitochondria has increased. Besides the absence of matrix granula, clearing of the matrix, and the onset of fragmentation of the cristae (→) occurs. Note intracellular oedema (*E*). Magn ×19600

Fig. 7. Severe ischaemic injury. Further destruction of mitochondria in form of increasing fragmentation of the cristae and clearing of the matrix has taken place. Magn ×5300

The ultrastructural appearance of each biopsy viewed by electron microscopy was documented by a number of photographs taken at lower (1000 ×) or higher (up to 20000 ×) magnification.

The degree of ischaemic damage in each biopsy was determined by evaluation with a semi-quantitative scoring system which we have used routinely for the evaluation of ischaemic damage in global and regional ischaemia during the past years (J. Schaper et al. 1979; J. Schaper 1980).

In this system changes of the myocardial cell nuclei and mitochondria, being the cellular organelles most sensitive to ischaemic damage, are graded in severity from being either absent (−) or present in a degree varying from (+) over (++) to (+++).

Changes in all other cellular organelles as well as the extracellular space were graded as being either absent (−) or present (+). In this manner a differentiated and reproducible evaluation of the degree of ischaemic injury can be obtained.

All biopsies were subsequently evaluated and determined as being of normal appearance or demonstrating slight, moderate or severe ischaemic damage, as reversible forms of ischaemic injury, or as irreversible ischaemic injury. The ultrastructural criteria have been well defined during the past years (Jennings et al. 1965, 1975; Jennings and Ganote 1976; Jennings 1976; J. Schaper et al. 1979; J. Schaper 1980).

Results

Each state of ischaemic damage and its characteristic changes are documented by Figs. 4–8 (photos).

The degree of ischaemic damage in every biopsy viewed is listed in the following Tables 1–4.

Table 1 summarizes the degree of ischaemic damage in subepi- and subendocardial sections of each biopsy taken after 90 min of ischaemic following control (C) or test (T) occlusion.

As shown in Table 1, after 90 min of CAO without HY-intervention (control occlusion) 3 out of 8 biopsies taken from the "normal tissue" showed signs of reversible ischaemic damage. The appearance of ischaemic damage in tissue taken from a normally perfused area will be discussed later in this report. Six out of 8 biopsies taken from the "infarct tissue" were irreversibly damaged, whereby all subendocardial tissues were irreversibly damaged and 2 transmural infarcts had occurred. These findings corresponded with the macroscopic infarct demonstration as performed by p-NBT staining.

After 90 min of CAO with HY-intervention (test occlusion) 2 out of 8 biopsies from the "normal tissue" showed signs of reversible ischaemic damage. In the "infarct tissue" no case of irreversible ischaemic damage had occurred. Subepi- and subendocardial tissues were damaged to a similar extent (Table 1).

Table 2 summarizes the degree of ischaemic damage in subepi- and subendocardial parts in each biopsy taken after 24 h of ischaemia without HY-intervention.

After 24 h of CAO without HY-intervention all biopsies from the "normal tissue" showed signs of reversible ischaemic damage.

Fourteen out of 16 biopsies from the "infarcted tissue" showed signs of irreversible ischaemic damage. Six transmural infarcts had occurred. These findings corresponded with the macroscopic dem-

Table 1. Ultrastructural results of group I (90 min occlusion)

Experiment No.	Occlusion time (min)	Normal		Infarct	
		epi	endo	epi	endo
1202	90 C	normal	slight/ moderate	normal slight	irreversible
1214	90 C	normal	normal	irreversible	irreversible
1266	90 C	normal/ slight	normal	slight	irreversible
1276	90 C	slight/ moderate	normal/ slight	irreversible	irreversible
1212	90 T	normal	normal	moderate	moderate
1225	90 T	normal	normal	moderate	moderate
1230	90 T	normal	normal	normal/ slight	normal/ slight
1252	90 T	normal	normal	slight	slight

Table 2. Ultrastructural results of group III (24 hours occlusion without HY-intervention)

Experiment No.	Occlusion time (h)	Normal		Infarct	
		epi	endo	epi	endo
973	24	slight	slight	irreversible	irreversible
1041	24	slight	slight/ moderate	irreversible	irreversible
1061	24	normal/ slight	slight	severe	irreversible
1085	24	normal/ slight	slight	irreversible	irreversible
1103	24	slight/ moderate	slight	irreversible	irreversible
1118	24	slight	slight/ moderate	severe	irreversible
1124	24	slight	slight	irreversible	irreversible
1231	24	slight	slight	irreversible	irreversible

Table 3. Ultrastructural results of group II (24 h occlusion with HY-intervention)

Experiment No.	Occlusion time (h)	Normal		Infarct	
		epi	endo	epi	endo
1388	24	normal	normal	moderate	irreversible
1399	24	slight/moderate	slight/moderate	irreversible	irreversible
1402	24	normal/slight	normal/slight	irreversible	severe/irreversible
1405	24	normal/slight	normal/slight	irreversible	irreversible
1441	24	normal/slight	normal/slight	irreversible	irreversible

Table 4. Percentage of irreversible ischaemic injury in HY-treated and control groups

	HY-treated	Control
90 min occl		
Epi	∅	50%
Endo	∅	100%
24 h occl		
Epi	80%	75%
Endo	100%	100%

Epi = Subepicardium; Endo = subendocardium; occl = occlusion

onstration as performed by p-NBT staining (Table 2).

Table 3 summarizes the degree of ischaemic injury in subepi- and subendocardial parts of each biopsy taken after 24 h of CAO with HY-intervention.

Eight out of 10 biopsies taken from the "normal tissue" showed signs of reversible ischaemic damage (Table 3).

Eight out of 10 biopsies taken from the "infarcted tissue" showed signs of irreversible ischaemic damage. Three transmural infarcts had occurred. These findings corresponded with the macroscopic infarct demonstration as performed by p-NBT staining.

Table 4 shows the percentage of irreversible ischaemic damage in tissue from the "infarcted" areas in treated versus control tissue after 90 min and 24 h of CAO as distributed in subepi- and subendocardial tissues.

Following 90 min of CAO the occurrence of irreversible ischaemic injury in tissue from "infarct" areas was 50% versus 0% for control versus HY-treated subepicardial tissue specimens, and 100% versus 0% for control versus HY-treated subendocardial tissue specimens.

After 24 h of CAO the distribution of irreversible ischaemic injury from "infarct" areas was 75% versus 80% for control versus HY-treated subepicardial tissue specimens and 100% for both control and HY-treated tissue specimens from the subendocardium.

Figures 9 and 10 show the membranes of a myocyte and an adjacent blood vessel as well as part of the interstitial space with and without exposure to HY. These figures serve as documentation of the observation that HY causes no ultrastructurally detectable changes of cellular membranes.

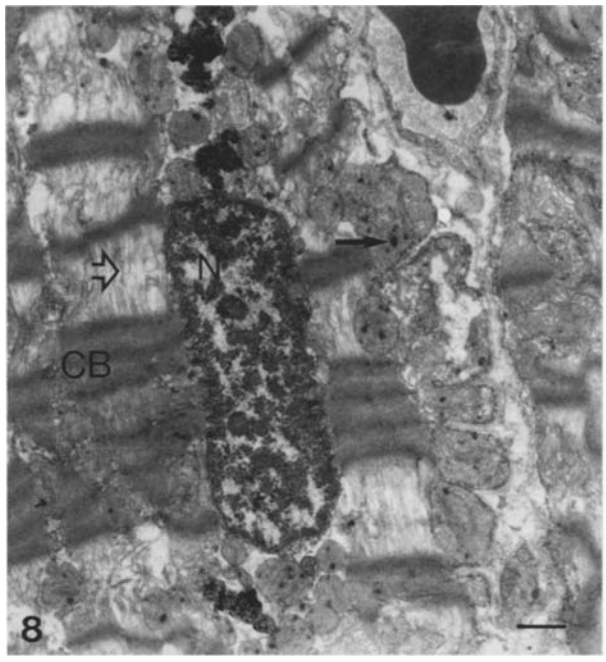
From these data it can be concluded that: 1. When administered before and during 90 min of CAO in the canine heart, intervention with HY prevents the development of irreversible ischaemic injury when compared with an untreated control group. 2. When performed before and during 24 h of CAO in the canine heart, HY-intervention cannot prevent the occurrence of irreversible ischaemic injury as compared with an untreated control group. 3. The development of irreversible ischaemic injury can therefore be delayed by HY-intervention. Progression to irreversible injury can, however, not be prevented.

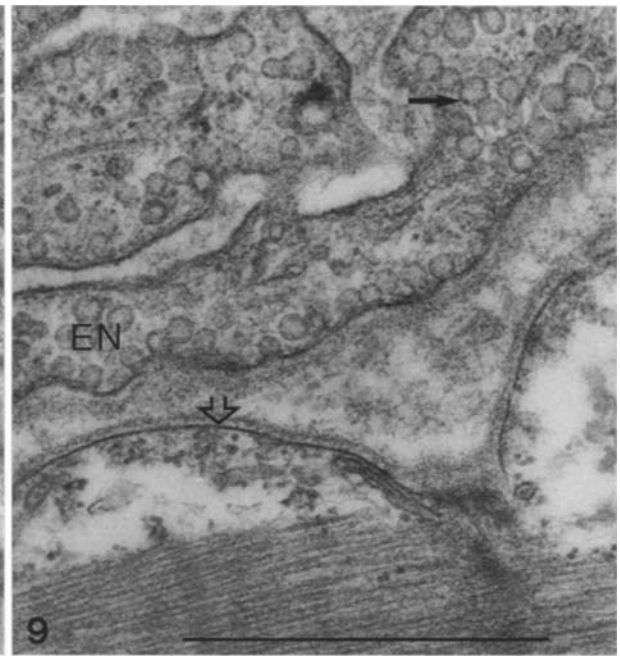
Discussion

In this study we have demonstrated that it is possible to delay the development of irreversible ischaemic injury in the canine heart during 90 minutes of ischaemia, by intervention with hyaluronidase. However, the factors determining progression of ischaemic injury to an irreversible stage is only influenced temporarily, since in this model irreversible ischaemic injury is present after 24 h of ischaemia to the same extent as if no intervention had taken place.

Our results are based on ultrastructural evaluation of tissue biopsies taken from the beating heart and are thus part of an in vivo system. Ischaemic damage in biopsies was graded by a semiquantitative scoring system.

This system was originally designed for the evaluation of effects of global ischaemia, later modified and further adapted to meet the study of regional ischaemia in this experimental model. In addition, the correlation of ultrastructural appearance and the functional state of nonischaemic and ischaemic myocardium had been established (J. Schaper et al. 1979). It was possible to match





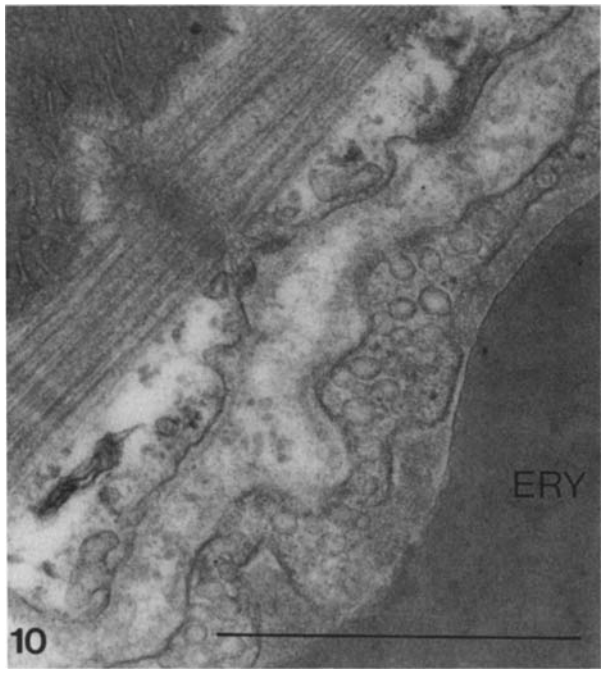


Fig. 8. Irreversible ischaemic injury. The mitochondria contain numerous electron dense "flocculent densities" (→) characteristic of irreversible ischaemic injury. The contractile material shows areas of hypercontraction (contraction bands – CB) and destruction (⇒). The nucleus (*N*) contains aggregated and clumped as well as marginated chromatin. Magn ×5300

Fig. 9. Normal membranes of myocyte and vascular endothelium without HY exposition. The vascular endothelium (*EN*) contains numerous pinocytotic vesicles (→). Note the distinct sarcolemma of the myocytes (⇓). Towards the interstitial space (*I*) the endothelium and myocytes are covered with a basal lamina. Magn ×48000

Fig. 10. Normal membranes of myocyte and vascular endothelium after HY application. Structures shown are the same as in Fig. 9. HY intervention causes no ultrastructurally detectable changes of the membranes of the vascular endothelium or the myocytes, nor of the interstitial space. The vascular lumen contains an erythrocyte (*ERY*). Magn ×48000

certain ultrastructural characteristics with functional states, thus allowing us to define certain degrees of ischemic injury.

Normal tissue subsequently suffers slight, moderate, and then severe ischaemic damage before undergoing irreversible ischaemic injury. The ultrastructural characteristics of each degree of ischaemic injury have been described in detail under Figs. 4–8.

Reversible ischaemic damage implies that after termination of ischaemia and initiation of ideal conditions, e.g. restoration of coronary blood flow, presence of adequate substrates etc., ultrastructure becomes normal. Even then, however, functional recovery of reversibly injured myocardium lags behind ultrastructural recovery (Mulch et al. 1979).

The occurrence of reversible ischaemic damage in tissue biopsies taken from a normally perfused area as mentioned in the results section, can be explained by two situations. First, in regional

ischaemia akinetic ischaemic tissue is surrounded by contracting myocardium which causes mechanical stress in the biopsy area. Second, when biopsies are taken from an area which has not suffered macroscopically visible ischaemic damage, this does not automatically exclude the possibility that reversible ischaemic injury of the subendocardium or subepicardium may have occurred, as only epicardial inspection has occurred.

Our results show that after 90 min of ischaemia without intervention irreversible ischaemic injury occurs more often in the subendocardial than in the subepicardial layers of myocardial tissue. This represents the well-known observation that ischaemic injury spreads from subendocardial to subepicardial layers of the myocardium. The occurrence of irreversible ischaemic injury can be prevented completely by intervention with HY since after 90 minutes of ischaemia with intervention not a single case of irreversible ischaemic injury occurred.

The situation is different after 24 h of ischaemia. Here, without treatment, irreversible ischaemic injury has spread into the subepicardial layers of the myocardium causing transmural myocardial infarction. Intervention with HY does not change this situation.

These ultrastructural findings are in agreement with the macroscopic infarct demonstration as performed with p-NBT and reported by Hofmann et al. (1980). Here, also, infarct size was reduced under HY-intervention during 90 minutes of ischaemia, but not, however, after 24 h of ischaemia.

In experiments comparable to ours (Afonskaia et al. 1977) preservation of myocardial ultrastructure has also been described, unfortunately, however, in a not very detailed manner. Our results differ from those of David et al. (1977), where myocardial ultrastructure apparently deteriorated after HY-intervention. It must be noted that a different experimental approach was chosen: HY-intervention was initiated 20 min after CAO and as a one-time-only bolus injection which is questionable when we consider that HY has a half-time of approximately 10 minutes, is deactivated by serum and is apparently ineffective in the presence of heparin (Wolf et al. 1982; 1984).

As mentioned in the introduction, a number of previous studies have indicated a beneficial effect of HY-intervention on myocardial metabolism and structure during ischaemia. However, the reason for the apparent delay of the development of irreversible ischaemic injury after intervention with HY remains unclear. Previous studies (Kones and Philips 1976; Szlavy et al. 1978; Wolf et al. 1981; Sunnergren and Rovetto 1983) have indicated that an influence on myocardial membranes and components of the interstitial space could be responsible. Stabilisation of membranes, by whatever means, could limit the damage caused by ionic imbalances accompanying ischaemia. These lead to the deterioration of membrane function and structure, thus causing a massive influx of extracellular fluid and ions into the cells initiating cellular death (Jennings et al. 1964; Whalen et al. 1974). Loss of cellular volume control can be viewed as a sign of irreversible injury (Jennings et al. 1974; Jennings 1976). We have analysed the ultrastructure of membranes of blood vessels and myocytes extensively and carefully in our tissue samples without finding any specific or unspecific changes under intervention.

Our results prove, in agreement with those of Hofmann et al. (1980) and MacLean et al. (1978) that the beneficial effect is only of temporary nature and occurs during the early stages of ischaemia. It is apparently lost in later stages, and irreversible ischaemic injury becomes inevitable. Additional intervention, could possibly prolong the time gained until, the ischaemic tissue is reached by actively growing collateral vessels (W. Schaper and Pasyk 1976).

At present, however, we can demonstrate the delay of the development of irreversible ischaemic injury following experimental coronary artery occlusion.

References

Afonskaia NI, Sharov VG, Nikolaeva LF (1977) Electron-microscopic study of changes in the myocyte ultrastructure under the effect of hyaluronidase in experimental myocardial infarct. Kardiologiia 17 (abstr):114–118

Braunwald E, Maroko PR (1976a) The use of hyaluronidase and hydrocortisone in the reduction of myocardial ischemia. Acta Med Scand (Suppl) 587:169–176

Braunwald E, Maroko PR (1976b) Effects of hyaluronidase and hydrocortisone on myocardial necrosis after coronary artery occlusion. Am J Cardiol 37:550–556

David H, Lindenau KF, Bohm J, Behrisch D, Wassilew G, Larnke H (1977) Qualitative and quantitative changes in the ultrastructure of the dog heart after temporary ischemia and coronary reperfusion. Exp Pathol 14:141–156

Hofmann M, Hofmann M, Schaper W (1980) Influence of hyaluronidase on infarct size following experimental coronary occlusion of short (90′) or long (24 h) duration. Basic Res Cardiol 75:340–352

Jennings RB, Sommers HM, Kaltenbach JP, West JJ (1964) Electrolyte alterations in acute myocardial ischemic injury. Circ Res 14:260–269

Jennings RB, Baum JH, Herdson PB (1965) Fine structural changes in myocardial ischemic injury. Arch Pathol 79:135–143

Jennings RB, Ganote CE (1974) Structural changes in myocardium during acute ischemia. Circ Res 34+35 (Suppl III):156–172

Jennings RB, Ganote CE, Reimer KA (1975) Ischemic tissue injury. Am J Pathol 81:179–198

Jennings RB, Ganote CE (1976) Mitochondrial structure and function in acute myocardial ischemic injury. Circ Res 38:(Suppl 1):180–191

Jennings RB (1976) Cell volume regulation in acute myocardial ischemic injury. Acta Med Scand (Suppl) 587:83–93

Kloner RA, Braunwald E, Maroko PR (1978) Long-term preservation of ischemic myocardium in the dog by hyaluronidase. Circulation 58:220–226

Kones RJ, Philipps JH (1976) Reduction in myocardial ischemia. Southern Med J 69:442–448

Maclean D, Fishbein MC, Maroko PR, Braunwald E (1976) Hyaluronidase-induced reductions in myocardial infarct size. Science 194:199–200

Maclean D, Fishbein MC, Braunwald E, Maroko PR (1978) Long-term preservation of ischemic myocardium after experimental coronary artery occlusion. J Clin Invest 61:541–551

Maroko PR, Libby P, Bloor CM, Sobel BE, Braunwald E (1972) Reduction by hyaluronidase of myocardial necrosis following coronary artery occlusion. Circulation 46:430–437

Maroko PR, Hillis LD, Muller JE, Tavazzi L, Heyndrickx GR, Ray M, Chiariello M, Distante A, Askenazi J, Salerno J, Carpentier J, Reshetnaya NI, Radvany P, Libby P, Raabe DS, Chazov EI, Bobba P, Braunwald E (1977) Favorable effects of hyaluronidase on electrocardiographic evidence of necrosis in patients with acute myocardial infarction. N Engl J Med 296:898–903

Mulch J, Schaper J, Scheld HH, Hehrlein FW (1979) Recovery of the heart after normothermic ischemia. Part I. Ultrastructural findings during postischemic reperfusion. Thorac Cardiovasc Surg 27:12–17

Oliveira JM, Carballo R, Zimmermann KA (1959) Intravenous injection of hyaluronidase in acute myocardial infarction: preliminary report of clinical and experimental observations. Am Heart J 57:712–722

Schaper J, Mulch J, Winkler B, Schaper W (1979) Ultrastructural, functional, and biochemical criteria for estimation of reversibility of ischemic injury: A study on the effects of global ischemia on the isolated dog heart. J Mol Cell Cardiol II:521–541

Schaper J (1980) Die Ultrastruktur des Myokards bei Ischämie. Habilitationsschrift, Universität Giessen, pp 48–96

Schaper W, Pasyk S (1976) Influence of collateral flow on the ischemic tolerance of the heart following acute and subacute coronary occlusion. Circulation 53 (Suppl I):57–62

Schaper W, Hofmann M, Müller KD, Genth K, Carl M (1979) Experimental occlusion of two small coronary arteries in the same heart. A new validation method for infarct size manipulation. Basic Res Cardiol 74:224–229

Schaper W, Frenzel H, Hort W (1979) Experimental coronary artery occlusion. I. Measurement of infarct size. Basic Res Cardiol 74:46–53

Sunnergren KP, Rovetto MJ (1983) Hyaluronidase reversal of increased coronary vascular resistance in ischemic rat hearts. Am J Physiol 245:H183–H188

Szlavy AL, Hollenberg NW, Abrams HL (1978) Cardiac lymph in normal and infarcted myocardium. Circulation 57 (Suppl II):36

Whalen DA, Hamilton DG, Ganote CE, Jennings RB (1974) Effects of a transient period of ischemia on myocardial cells. Effect on cell volume regulation. Am J Pathol 74:381–397

Wolf RA, Chaung LY, Muller JE, Kloner RA, Braunwald E (1981) Intravenous bovine testicular hyaluronidase depolymerizes myocardial hyaluronic acid in dogs with coronary artery occlusion. Circ Res 48:88–94

Wolf RA, Chaung LY, O'Hara D, Smith TW, Muller JE (1982) The serum kinetics of bovine testicular hyaluronidase in dogs, rats and humans. J Pharmacol Exp Ther 222:333–337

Wolf RA, Glogar D, Chaung LY, Garrett PE, Ertl G, Tumas J, Braunwald E, Kloner RA, Feldstein ML, Muller JE (1984) Heparin inhibits bovine testicular hyaluronidase activity in myocardium of dogs with coronary artery occlusion. Am J Cardiol 53:941–944

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