Contraction in cardiac endothelial cells contributes to changes in capillary dimensions following ischaemia and reperfusion

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Received 22 March 2000; accepted 29 June 2000

Abstract

Objective: Ischaemia followed by reperfusion brings about a reduction in cardiac capillary cross-sectional dimensions which is consistent with constriction. The aim of this study was to test the hypothesis that the reduction in cardiac capillary dimensions that occurs in ischaemia and reperfusion is caused by endothelial cell contraction and that modulating the endothelial cell contractile apparatus reduces microvascular reperfusion injury. Methods: In isolated rat hearts we used phalloidin to stabilise the endothelial actin filaments in order to prevent the dimensional changes during ischaemia. The changes in endothelial cell dimensions were quantified by measuring whole capillary and luminal cross-sectional areas, abluminal and luminal membrane lengths. We have also used resin casts of the coronary vasculature coupled with scanning electron microscopy to examine the structural changes along the length of the capillaries in ischaemia–reperfusion. Results: We found that the reduction in capillary dimensions was prevented by the addition of phalloidin and, in the resin casts, that ischaemia–reperfusion cause focal narrowings along the capillaries which are consistent with constriction. Conclusions: (1) The endothelial contractile apparatus is involved in the reduction in cross-sectional dimensions. (2) This implies that the capillary bed may have a greater role in the local control of flow than was previously thought and that modulation of the actomyosin contractile system in cardiac capillary endothelial cells may be useful in reducing ‘no reflow’ injury which results from reperfusion. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Capillaries; Contractile apparatus; Contractile function; Electron microscopy; Endothelial function; Ischaemia–reperfusion

1. Introduction

Regulation of blood flow in the microvasculature is effected predominantly by arteries, arterioles and precapillary sphincters. Pericytes, distributed periodically along the length of capillaries, are able to induce constriction in capillaries by contraction of cell processes which partially envelope the capillary [1]. Active capillary constriction has been shown to occur in the pancreas [2], rete capillaries [3] and the spleen [4]. In the heart, however, the concept of active capillary constriction, per se, remains controversial. Cardiac capillary cross-sectional area has been shown to be reduced during hypoxia and ischaemia without endothelial cell swelling [5,6]. On reperfusion the reduction in total cross-sectional area is coupled with swelling of the endothelial cells [6]. This change in capillary dimensions may be the result of myocyte contraction or oedema or it may be due to capillary constriction induced by activity of the actomyosin contractile apparatus in the endothelial cells. The elements and viability of a contractile system in endothelial cells have been demonstrated by several researchers [4,7–9]. Recent work indicates that a contractile mechanism is associated with transendothelial cell gaps in rats, guinea pigs, and mice [10]. In addition, induction of endothelial cell membrane blebbing as occurs on reperfusion, by peroxide generated radicals, is associated with actin polymerisation [11]. The question of whether cardiac capillary endothelial cells (CCECs) are actively involved in narrowing of the capillary lumen is significant in that

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PII: S0008-6363(00)00173-5
their participation in this process may contribute to reperfusion injury and the ‘no reflow phenomenon’. This post-ischaemic microvascular incompetence is the inability of the microvasculature to supply a region of the myocardium with blood following ischaemia [12–14] and leads to regional myocyte death. Reperfusion injury is recognised as the damage caused by reperfusion alone over and above that caused by ischaemia [15,16].

Indirect evidence suggesting that capillary endothelial cells contribute to capillary constriction is considerable [7,17,18]. It includes evidence that many vasoactive chemicals induce reorganisation of the endothelial microfilament system and cell surface area [19–21]. Intervention in the contractile system, using a chemical tool, known to specifically target a major component of the contractile apparatus, would provide more direct evidence of the contractile activity of endothelial cells. It would modulate any contraction which would normally result from the activity of the contractile apparatus. The phal- lotoxin, phalloidin, is a suitable candidate for this purpose because it targets actin specifically to stabilise microfilaments [22,23]: it modulates the actomyosin system in endothelial cells by stabilising F actin. Phalloidin has been shown not to affect endothelial cell viability, growth or metabolism [19,20,24].

The present report supports the conclusions of these preceding studies and demonstrates that reperfusion injury in the ischaemic–reperfused heart is significantly influenced by the endothelial contractile apparatus. We have used phalloidin to demonstrate that, by modulating the actomyosin contractile system in CCECs in a live heart, the dimensional changes which normally occur following ischaemia and reperfusion in viable endothelial cells are prevented. We have compared morphometric measurements following reperfusion in CCECs perfused with and without phalloidin. In order to gain insight into possible constrictions in the longitudinal dimensions of the capillaries, we have also examined resin casts of the cardiac microvasculature.

2. Methods

This investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.1. Perfusions

Hearts were excised from male Wistar rats, approximately 250 g in weight, which had been killed by cervical dislocation. They were immersed immediately in ice cold oxygenated Krebs–Henseleit solution containing 5 mM glucose and 0.2 mM albumin. The perfusate was oxygenated by bubbling with 95% O₂–5% CO₂ (pO₂ ~95 kDa). The aorta was cannulated and the coronary circulation perfused using a non-recirculating Langendorff preparation. The perfusate was maintained at a flow-rate of 10 ml min⁻¹ g⁻¹ at 37°C. All experiments began with an equilibration period of 15 min. Perfusion pressure in the aorta was measured constantly via a pressure transducer.

All experiments involving phalloidin and morphometry lasted 110 min. Four categories of experiment (six hearts in each group) were carried out as follows:

1. Control experiments where the hearts were constantly perfused (without phalloidin) for 110 min, then fixed with a 4% formaldehyde and 0.25% glutaraldehyde in phosphate buffered saline.
2. Control experiments where the hearts were constantly perfused, for 15 min with solution without phalloidin followed by another 95 min with 10⁻⁶ M phalloidin solution. The hearts were then fixed as above.
3. After the 15-min equilibration period hearts were perfused without phalloidin for 50 min. Turning the perfusion pump off then induced an ischaemic period of 45 min. This was followed by reperfusion for 15 min without phalloidin and then formaldehyde fixation of the hearts.
4. The hearts were perfused normally for 15 min with solution without phalloidin followed by 35 min with solution containing phalloidin. The pump was then switched off to induce an ischaemic period of 45 min. After this the pump was restarted to reperfuse the heart for 15 min with phalloidin solution. The hearts were then fixed.

2.2. Transmission electron microscopy

In all experiments, fixation was accomplished by exchange of the perfusate for the fixative, 100 ml of which was passed through the coronary circulation. Tissue samples, 1 mm², were taken from the free wall of the left ventricle, mid-myocardial region and allowed to fix in the same fixative for 4 h at 4°C. These were then fixed for 1 h in 2.5% glutaraldehyde, embedded in epoxy resin (Araldite) and processed for electron microscopy. Three blocks from each heart were sectioned and from 4 to 7 images of capillary cross-sections were captured from two nonconsecutive sections from each block. For each group, the number of capillary images captured was as follows: control 116, reperfused 108, control with phalloidin 106 and reperfused with phalloidin 102.

2.3. Resin casts and scanning electron microscopy

All experiments for resin casts endured for 60 min and
did not include reperfusion. Two categories of experiments were carried out.

1. Control hearts (n = 4) were perfused for 60 min and then fixed with formaldehyde/glutaraldehyde buffer which had been bubbled with nitrogen for the 30 min preceding its use to remove oxygen. Resin casts were made by perfusing a methyl methacrylate resin (Baton’s no. 17) into the heart immediately following fixation. After 100 ml of fixative had passed through the heart, freshly mixed resin was pumped into the microvasculature using pump settings identical to those used during the earlier perfusion. Once ≈2 ml of resin had passed out of the heart into a collecting vessel, the pump was switched off. The heart was left in water at room temperature for at least two h to allow complete polymerisation of the resin. The heart was then alternately changed from a solution of sodium hydroxide to distilled water, allowing 24 h between each change. Once all tissue had been digested away, the cast was dried, gold coated and examined in a scanning electron microscope.

2. Ischaemia was induced in four hearts by switching the pump off after 15 min of perfusion. Following an ischaemic period of 45 min the hearts were fixed immediately with anoxic fixative. Resin infiltration was performed as described above.

2.4. Morphometric measurements

Images were grabbed from the electron microscope using a TCIpro image grabbing system and saved on a dedicated PC. Morphometric analysis was performed using a software package, IMAGE PRO VERS. 3.1. Using this, the following parameters were measured: cross-sectional area of capillaries, lumen and endothelial cells, as well as luminal and abluminal perimeters. Accuracy was assured by calibration of both the electron microscope and the image analysis system with graticules of known dimensions. A roundness factor allocated to each capillary, where a perfectly round capillary was ascribed a factor of 1. Measurements of capillaries with a roundness factor of greater than 2.1 were discarded. The final number of capillary images used in morphometric analysis of each group was as follows: control 112, reperfused 106, control with phalloidin 91 and reperfused with phalloidin 91.

2.5. Statistics

Statistical analysis was carried out using EXCEL 5 and the SPSS analysis package vers. 10. A one-way ANOVA (analysis of variance) with Bonferroni post-hoc tests for unplanned comparisons was carried out using the measurements which had been corrected for obliqueness as described by Ward and McCarthy [6]. When P values were less than 0.05, statistical significance was considered reached. Results are presented in Table 1.

3. Results

3.1. Resin casts

The variation displayed by capillaries which have been well oxygenated is demonstrated in typical images of corrosion casts in Fig. 1. A low magnification of the capillaries is shown in Fig. 1A. The branching capillaries are relatively regular and cylindrical, occasionally with some sphincter like constrictions. Higher magnification (Fig. 1B–E) shows little variation in capillaries with few narrowed areas. However, some capillaries did demonstrate constrictions as shown in Fig. 1D. The topographical impression of the lumen wall is rough but regular. A high magnification of this surface area, Fig. 1E, illustrates the typical endothelial lumen surface regularity of the capillaries in a well oxygenated heart.

The effect of a long period of ischaemia on capillary endothelial cells can be seen in Fig. 2. Capillaries have lost the regularity seen in well oxygenated capillaries (Fig. 1) and tended to be more convoluted. In addition, an area of ‘no reflow’ can be seen in Fig. 2A with blunt ending capillary casts projecting into open void. Constriction points were more prevalent while a second type of constriction or narrowing, not observed in well oxygenated capillaries, developed in ischaemic capillaries. Examples of this type of constriction are shown in Fig. 2C–I. The lumen casts shown, contrast with images (at a similar magnification) of lumen casts in oxygenated hearts, a typical example of which is shown in Fig. 1E. The topography of the luminal surface area is considerably more rugged and irregular, often with the lumen calibre reduced at a particular point, as in Fig. 2G, to a much greater degree than seen in constriction sites in capillaries.
Fig. 1. Resin casts of the small vessels in a rat heart, perfused for 60 min. A, B, C and E represent capillary lumina (with a diameter of up to 8 μm) while D may be a postcapillary venule. These capillaries have a coarse but regular luminal surface with a generally circular circumference. This contrasts with the luminal surface topography of resin casts of ischaemic capillaries. Bar=μm.
Fig. 2. Resin casts of the small vessels in a rat heart, following ischaemia for 45 min (after perfusion for 15 min with oxygenated buffer). The absence of lumen casts in (A) in the area to the left of centre indicates a zone of no reflow. In comparison with well oxygenated capillaries, most of the luminal surfaces are more irregular and many demonstrate a more angular circumference as in (F–I). In addition, ridges are present, particularly at sites where the lumen is constricted (F–I). The severely constricted capillary lumen, as shown in (E) and (G) occurs in ischaemic preparations but not in normally perfused hearts. (F–I) illustrate lumen dimensions which are only observed in ischaemic hearts [a single loop in lower right of (C) and a higher magnification shown in (C). Bar=µm.
in oxygenated hearts. The topography demonstrated in Fig. 2F–I was not found in casts of well oxygenated capillaries. These images reveal that each capillary lumen was the subject of some degree of constriction with that shown in Fig. 2G being the most severe.

3.2. Capillary cross-sections

Fig. 3A depicts a typical cardiac capillary, in cross-section, which has been perfused routinely with an oxygenated perfusate without phalloidin, for 110 min before fixation. The endothelium is of a relatively uniform width and the cytoplasm contains many vesicles and caveolae. The myocardium is well preserved. In each case the heart continued to beat until it was fixed and perfusion pressures were below 70 mmHg. The lumen cross-sectional area is large compared with that of the narrow capillary walls. Another set of experiments was carried out to demonstrate that the addition of phalloidin to the perfusate did not result in any significant changes to the capillary dimensions in comparison with dimensions of the control capillaries (Fig. 3B) neither was myocyte structure affected. Control hearts (i.e. not ischaemic–reperfused) which were perfused with phalloidin similarly continued to beat until they were fixed.

The typical morphology of an ischaemic/reperfused capillary without phalloidin is shown in Fig. 3C. The capillary dimensions have altered considerably; the ratio of the luminal cross-sectional area to capillary wall (endothelial cell) area has decreased. In this capillary, in which

![Fig. 3. Electron micrographs of transverse sections of cardiac capillaries perfused for 110 min which demonstrate the protective effect of phalloidin. Each capillary cross-section consists of a single endothelial cell. The two capillaries shown in (A) and (B) have been perfused constantly with well oxygenated medium which, in the case of (B) also contained phalloidin. Similarly both the capillaries in (C) and (D) were perfused for 50 min before being subjected to ischaemia for a further 45 min. Only (D) however, was perfused with medium containing phalloidin. The ratio of cross-sectional area of the endothelial cell to luminal area in (C) is significantly greater than in any of the other cells shown in this figure. Bar=μm.](https://academic.oup.com/cardiovascres/article-abstract/48/2/346/331129)
the CCECs have undergone moderate swelling, the luminal diameter decreased significantly, while the diameter of the capillary changed little. The myocytes show typical reperfusion damage with disrupted myofibrils and swollen mitochondria with disrupted cristae.

The modulating effect of phalloidin is demonstrated by the image in Fig. 3D. The capillary and endothelial cell cross-sections retained the proportions typical of control capillaries that have not been subjected to ischaemia. In this last set of experiments, ischaemic capillaries were reperfused with the addition of phalloidin to the perfusate. The morphology of the endothelium is similar to that seen in the control endothelium. The cytoplasm is dense and contains organelles, caveolae and vesicles while the endothelium is of a similar thickness to that seen in control endothelium. The ratio of the endothelium to luminal cross sectional areas is also similar. Myocyte structure is well preserved and is similar to control hearts.

3.3. Morphometry

The results of the morphometry are shown in Fig. 4 with statistical values in Table 1.

1. Endothelial cell area: endothelial cell area was increased in ischaemic-reperfused capillaries from
2.0 ± 0.08 to 2.91 ± 0.17 μm². This increase was reversed when phalloidin was included in the perfusate and cell area fell below control values at 1.74 ± 0.1 μm². Controls with and without phalloidin were not significantly different.

2. Whole capillary area: phalloidin, without ischaemia–reperfusion reduced capillary area from 11.97 ± 0.5 to 9.47 ± 0.8 μm². There was, however, no significant difference between the controls with phalloidin and ischaemic–reperfused with phalloidin (9.47 ± 0.8 compared with 8.41 ± 0.7 μm²). This is in contrast to the large reduction in capillary area between controls without phalloidin 11.97 ± 0.5 μm² and ischaemic reperfused without phalloidin 7.39 ± 0.54 μm².

3. Luminal area: the luminal area measurements gave similar results. Phalloidin alone reduced the luminal area but the large reduction in luminal area from 9.77 ± 0.2 μm² in controls without phalloidin to 4.49 ± 0.4 μm² in ischaemic–reperfused without phalloidin did not occur in phalloidin perfused hearts (7.06 ± 0.7 μm² in controls with phalloidin, 6.42 ± 0.6 μm² in ischaemic–reperfused with phalloidin). The luminal area was significantly greater in ischaemic–reperfused capillaries with phalloidin than without.

4. Perimeters: phalloidin reduced both capillary and luminal perimeters. The capillary perimeters of all ischaemic–reperfused groups were not significantly different from each other (10.84 ± 0.4, 10.18 ± 0.36 and 10.04 ± 0.4 μm, respectively) but were significantly different from the control (12.90 ± 0.3 μm). The luminal perimeter, however, was smaller in ischaemic–reperfused capillaries without phalloidin than in ischaemic–reperfused capillaries with phalloidin (8.32 ± 0.38 μm compared with 9.34 ± 0.37 μm) but not significantly different.

4. Discussion

4.1. Period of ischaemia

This study was designed to determine whether the reduction in capillary dimensions seen in ischaemia–reperfusion might be due to contraction of the constituent endothelial cells. An ischaemic period of 45 min was chosen for both the resin cast experiments and the experiments with phalloidin in an attempt to ensure that some capillaries sustained irreversible damage while in others the damage was reversible. Damage sustained by CCECs during ischaemic insult varies considerably. Ward and McCarthy [6] demonstrated that most damage sustained by endothelial cells following ischaemia for 30 min is reversible but following 60 min of ischaemia most of the damage is not reversed by reperfusion. An ischaemic period of 45 min, midway between these two time points was therefore chosen in order to avoid both these extremes.

4.2. Resin casts

Morphometric measurements of cardiac capillary cross-sections have shown a reduction in capillary dimensions in ischaemia that is coupled with endothelial cell swelling on reperfusion. This method however, does not provide any insight into the longitudinal dimension of capillaries, on the regularity of occurrence of possible constrictions or whether they are discrete narrowings or are more gradual ones occurring over relatively large sectors of capillaries. The latter would suggest compression by myocyte blocks whereas sudden narrowing, spanning a relatively short capillary length, in a sphincter like manner, would support constriction. The resin casts demonstrated that, in capillaries subjected to ischaemia, and those which were well oxygenated (controls), capillary diameter reductions were abrupt rather than gradual, suggesting constriction, rather than myocyte compression or oedema.

Resin casts of reperfused hearts contained spaces devoid of any capillary casts. Since these voids were within the capillary meshwork well away from its edges, and a similar void could not be found in the normally perfused (control) samples, it is unlikely that this is the result of specimen processing. These voids represent zones of no reflow which occur on reperfusion.

Capillary constriction has not been recorded in continuous capillaries. Ragan et al. [4], however, made visual recordings of contractions in normal capillaries in the rat and mouse spleen. These capillaries were shown to narrow the lumen at points along their length by changing the shape of the endothelial cells. They did not observe any ‘plugging’ of the lumen, when the calibre of the lumen was significantly reduced but the passage of individual red blood cells through the constriction was slowed down considerably. At any one time 1 in 12 and 1 in 58 capillaries were contracting in mice and rats, respectively. These changes in capillary calibre coincided with an increase in the width of the corresponding endothelial cells. Ragan et al. [4] demonstrated that cyclical changes in spleen capillaries were the result of endothelial contraction and not caused by the contraction and relaxation of capsular and trabecular smooth muscle. This would have caused a narrowing of a capillary along its length rather than at specific points as they observed in their experiments. Similarly, we suggest that the point constrictions seen in our resin casts of capillary lumina are caused by CCEC contractions and not by pressure exerted on capillaries by compression from myocyte blocks or oedema causing transmural variations. This would have resulted in more gradual changes in lumen membrane contours than those we observed.

We have described two types of constriction in resin casts, one of which was observed in both ischaemic and well oxygenated hearts while the other was observed almost exclusively in ischaemic hearts. In well oxygenated hearts, the branching capillaries are relatively regular and
4.3. Pericyte activity

The potential of pericytes to constrict capillaries is recognised [25]. In the heart preparations, capillaries with morphology consistent with constriction produced by pericyte activity, i.e. where constricted capillaries maintained their relatively cylindrical dimensions (an example of such a constriction is shown in Fig. 1D) were only occasionally seen. The significant increase, however, in constrictions in ischaemic capillaries was coupled with a significant change in the surface area and dimensions of many of the capillaries. Where they were constricted, these capillaries lost their cylindrical appearance and adopted shapes with lumen perimeters containing irregular angles and depressions. These constrictions were not sphincter-like in appearance as would be expected in the case of narrowing caused by pericytes. It is more likely that these characteristics were induced by localised changes in endothelial cell dimensions. In reperfusion experiments on pig heart capillaries, Pathi et al. [26] noted that, although reperfused capillaries recovered from a prolonged period of ischaemia, they did not revert to their preischaemic calibre. Electron microscopy indicated that these capillaries were not subjected to cellular or interstitial oedema; Pathi et al. were unable to account for this residual narrowing of the capillaries. We suggest that the endothelial cells were maintaining this narrowed capillary diameter by active contraction.

For several reasons, it is unlikely that pericytes were responsible for all of the constrictions observed. Egginton et al. [27] reported pericyte coverage per capillary, in the heart, as 11%, a ratio of 1 pericyte for every 9 endothelial cells. This relatively regular distribution of pericytes suggests that the pattern of constriction seen in both heart groups, but particularly in the ischaemic group, would be more regular and frequent than that observed in our studies. The concept of capillary ‘narrowings’ induced by endothelial cells rather than pericytes is not new. Ragan et al. [4], demonstrated capillary ‘narrowings’ in visual recordings of normal capillaries contracting in the rat and mouse spleen, where pericytes were not in the proximity of the endothelial cells involved in the contraction.

Aharinejad et al. [2] observed, in pancreas, two types of capillaries, fenestrated and non-fenestrated. In the non-fenestrated type, pericytes did not appear to play a part in the cyclical constrictions. Similarly, pericytes associated with continuous capillaries in the rat heart are reported to have only a vestigial contractile potential [25]. These pericytes contrast with those in rat skeletal muscle which are contractile and associate with elaborate endothelial processes. This association does not occur in cardiac pericytes. Secondary pericyte processes, tentacle-like cell extensions which extend out of and away from the cell body to enclose the associated capillary, present in rat skeletal muscle, are rare, if not absent, in rat myocardium [25]. The circumferential coverage of capillaries by cardiac pericytes is half that of skeletal muscle pericytes. In addition, rat cardiac pericytes are not affected by most vasoactive substances while skeletal pericytes react strongly to the same agents [27].

4.4. Morphometry

Ward and McCarthy [6] demonstrated that during ischaemia, although the cross-sectional area of endothelial cells remains the same, total capillary diameter decreases significantly but the luminal diameter decreases to a greater extent. In the present study, without phalloidin, there was a considerable decrease in the luminal perimeter in the ischaemic–reperfused capillaries compared with control capillaries. In passive capillary constriction, to comply with the reduced area, the perimeter would not be reduced in length but would be rearranged into a convoluted pattern around the lumen (a premise also referred to by Tilton et al. [28]). Our observations, however, demonstrated that after the reduction in luminal area, the luminal perimeter was reduced in length and regular in appearance. This suggests that superficial luminal membrane was absorbed or extruded as blebs in our experiments. No membrane convolutions were observed. Blebs were only observed in the most severely damaged cells. This luminal perimeter adjustment was, therefore, consistent with contraction rather than an external influence such as compression by myocyte blocks. The absence of convolutions in the luminal membrane indicates that the superficial membrane is internalised within the cell by resorption and membrane dissolution.

4.5. Phalloidin inhibits endothelial cell shape change in ischaemia–reperfusion

By incorporating phalloidin into the perfusion medium, the extreme dimensional rearrangements which occur during reperfusion, as shown by Ward and McCarthy [6], may be attenuated while maintaining the viability of the myocardium as evidenced by the continued beating of the heart and the well preserved ultrastructure. The phalloidin did have some effect per se on endothelial cell shape without ischaemia–reperfusion in that the capillary and luminal area were reduced. Phalloidin, however, protected CCECs from reperfusion injury by preventing the significant increase in endothelial cell area which accompanies reperfusion following ischaemia. This is consistent with the studies reported by Alexander et al. [24] and Rasio et al. [20]. Rasio et al. [20] exposed continuous and fenestrated capillaries in an ex vivo preparation of the eel bladder rete vascular system to phalloidin for considerably longer periods than were used in our experiments. They used the same concentration of phalloidin as we used.
(without ischaemia or reperfusion) and reported that the endothelial cells did not display any morphological differences in comparison with normally perfused control endothelial cells not subjected to phalloidin [20,29]. Alexander et al. [24] demonstrated that phalloidin reduced endothelial barrier permeability, and reported that phalloidin induced cell shape change: cell flattening and spreading. They concluded that phalloidin reduces permeability to albumin and modulates oedema by its influence on the cytoskeleton. These experiments, however, were in vitro. In skeletal muscle, Korthuis et al. [30] demonstrated significant inhibition of changes in microvascular permeability induced by ischaemia–reperfusion by phalloidin via its effect on the microfilament system of endothelial cells. Alexander et al. [24] suggested that therapies to control oedema should centre on agents (such as phalloidin) which affect the cytoskeleton directly. We similarly suggest that such agents, without the toxic side effects of phalloidin, for example cell specific kinase inhibitors, may prove useful in modulating reperfusion injury and ‘no reflow’ by controlling cell shape change during ischaemia and reperfusion.

Damage imposed by toxic reactive oxygen species, which are released during ischaemia–reperfusion [31] impinge on the microfilament system [32–34]. We have shown that stabilisation of the microfilament system, prevents or ameliorates the damage caused by these reactive oxygen metabolites. Clearly, evidence supporting a strategy of intervention at the level of the microfilament system, as a therapeutic avenue to regulate reperfusion injury and, possibly, ‘no reflow’, is strengthening.

No reflow following ischaemia and reperfusion is probably the cumulative result of several potential factors, for example, the release of cytokines and free radicals, leukocyte plugging of capillaries and myocyte contraction [15,35–37]. This study has has provided more evidence for the active involvement of CCECs, by altering their shape, in the changes in capillary dimensions which occur during reperfusion and the no reflow phenomenon. More importantly, we have demonstrated that changes in CCECs may be modulated by targeting the acto-myosin system and that this may be a useful strategy in reducing injury caused by reperfusion and, by implication, the ‘no reflow’ phenomenon.

Acknowledgements

This study was supported by the British Heart Foundation.

References


