MORPHOLOGY AND PATHOMORPHOLOGY

Heat Shock Protects Endotheliocyte Actin Cytoskeleton from Destruction under Conditions of ATP Depletion: Role of Heat Shock Protein HSP27

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Heat shock followed by 12-18-hour recovery induces tolerance of cultured endothelial cells to ATP depletion preventing degradation of actin filaments and HSP27 dephosphorylation/redistribution. HSP27 accumulates during this period, while inhibition of protein synthesis abolishes the protective effect of heat shock.

Key Words: *endothelium; actin cytoskeleton; heat shock protein HSP27; ATP depletion; heat shock*

Structural abnormalities of endothelial cells (EC) in ischemia, primarily destruction of actin cytoskeleton, increases capillary permeability for macromolecules and blood cells. Endotheliocyte dysfunction in acute ischemia is a result of their energy depletion caused by inadequate ATP synthesis due to substrate and oxygen deficiency. ATP is an important regulator of actin polymerization/depolymerization in cells, and ATP depletion leads to a decrease in the intracellular Ca^{2+} concentration and microfilament disintegration [3,6]. On the other hand, heat shock (HS) protein HSP27 affects the time course of actin polymerization. It can inhibit actin polymerization by capping the "feathered" ends of the polymer [13]. Actin polymerization and microfilament resistance to HS, oxidants, and cytochalasin D depend on HSP27 phosphorylation and structure [5,11]. Preliminary HS induces cell and tissue tolerance to subsequent ischemia [9], but molecular mechanisms of this phenomenon are not clear. Increased expression of HSP27 protects the actin cytoskeleton in mammalian cells from HS, oxidants, and cytochalasin D [7,10,11].

We studied the relationship between HS-induced accumulation of HSP27 in cultured human vascular EC and the behavior of the actin cytoskeleton in ischemia-simulating metabolic stress.

MATERIALS AND METHODS

Epitheliocyte cultures (passages 0-2) from human aorta or umbilical vein [1] grown on slides or plastic were subjected to HS (45°C for 10 min) followed by recovery under standard conditions (after replacing the medium in cultures). ATP depletion was induced by incubation of EC in glucose-free DMEM containing 20 μM carbonylcyanide m-chlorophenylhydrasone (CCCP, uncoupler of oxidative phosphorylation) or 20 μM rotenone (mitochondrial respiratory chain inhibitor). The content of ATP in EC was measured by the luciferin-luciferase method for adhesive cells [8]. Intracellular Ca^{2+} was measured by Ca^{2+} fluorescent probe

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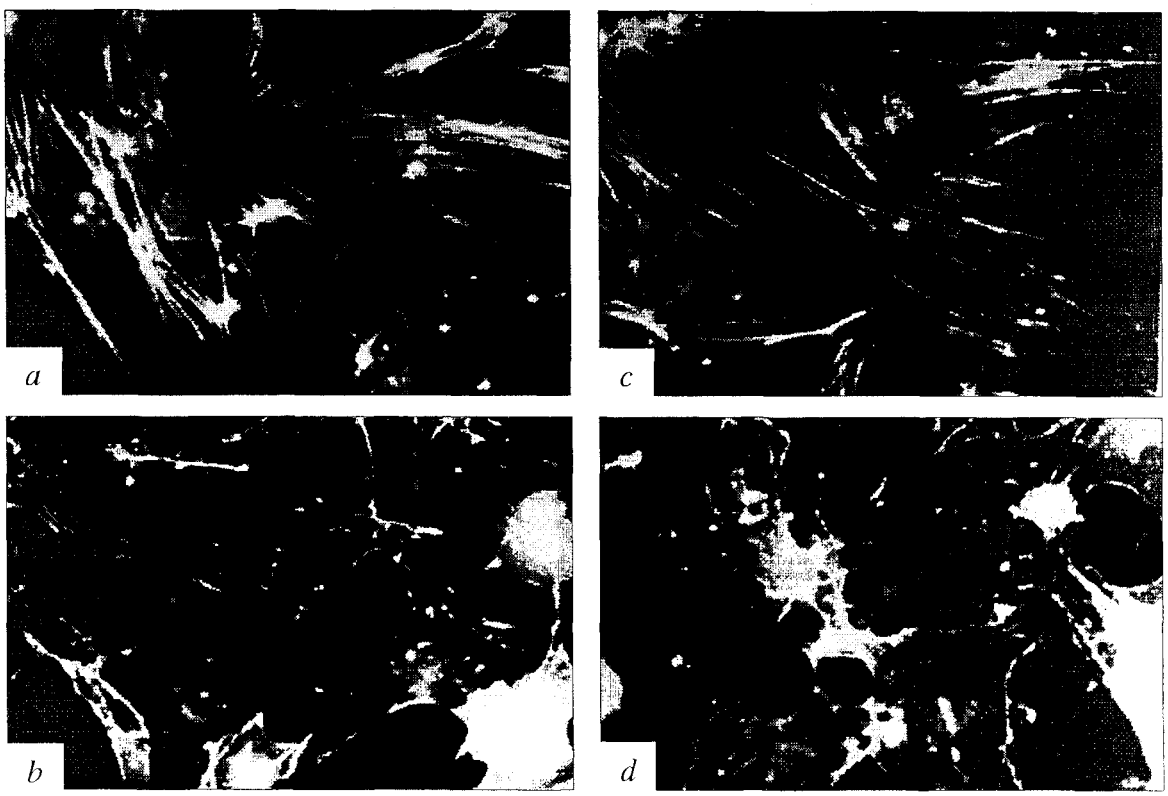

Fig. 1. Visualization (*a*), destruction (*b*), and protection (*c*) of F-actin. Here and in Fig. 2: *a*) non-stressed cells; *b*) after 2-h ischemia-simulating metabolic stress; *c*) the same, in tolerant endothelial cells; *d*) quercetin inhibition of protective effect of thermal pretreatment. Fluorescent microscopy, ×400.

Fura-2. Immunofluorescent analysis was carried out after fixation of cells in a mixture of 3.7% formaldehyde and 0.1% triton X-100 with anti-HSP27 antibodies (a gift from M. Gaestel) and second antibodies conjugated with Texas red (South Biotech. Assoc.). Tetramethylrhodamine isothiocyanate-conjugated phalloidin (Sigma) was used for fluorescent detection of F-actin. SDS electrophoresis was carried out in the Laemmli's system with 4% concentrating and 15% separating gels, immunoblotting was performed with antiHSP27 by the ECL method. HSP27 isoforms were identified by isoelectric focussing and immunoblotting with antiHSP27 [14]: the cells were lyzed in 8 M urea containing 1% Nonidet P-40, 2% β-mercaptoethanol, and 100 μM Na_3VO_4. Isoelectric focussing was carried out at pH 5-7, after which the proteins were transferred to a nitrocellulose filter and immunoblotting was performed.

RESULTS

Previously we showed that ATP depletion in EC induced disintegration of actin filaments and HSP27 dephosphorylation and translocation from the cytoplasm to the nucleus with the formation of granules insoluble in Triton X-100 [12]. Now we found that HS followed by 12-18-hour recovery induced EC tolerance to metabolic stress simulating ischemia (20 μM CCCH, 2 h).The cells retained regular structure, which was due to the integrity of actin cytoskeleton (Fig. 1, *a-c*). The structure and intracellular location of HSP27 also remained unchanged (Fig. 2, *a-c*). Iso-

TABLE 1. Concentration of ATP and Intracellular Ca^{2+} in Cells before and after Ischemia-Simulating Metabolic Stress (*M±m*)

Parameters		Time, h	Initial value		
			0.5	1	2.5
ATP, %	control EC	100	33±5	14±3	5±1
	tolerant EC	100±10	32±5	14±4	5±1
Ca^{2+}, nM	control EC	65±7	500±4	650±70	750±80
	tolerant EC	60±6	520±70	660±70	750±85

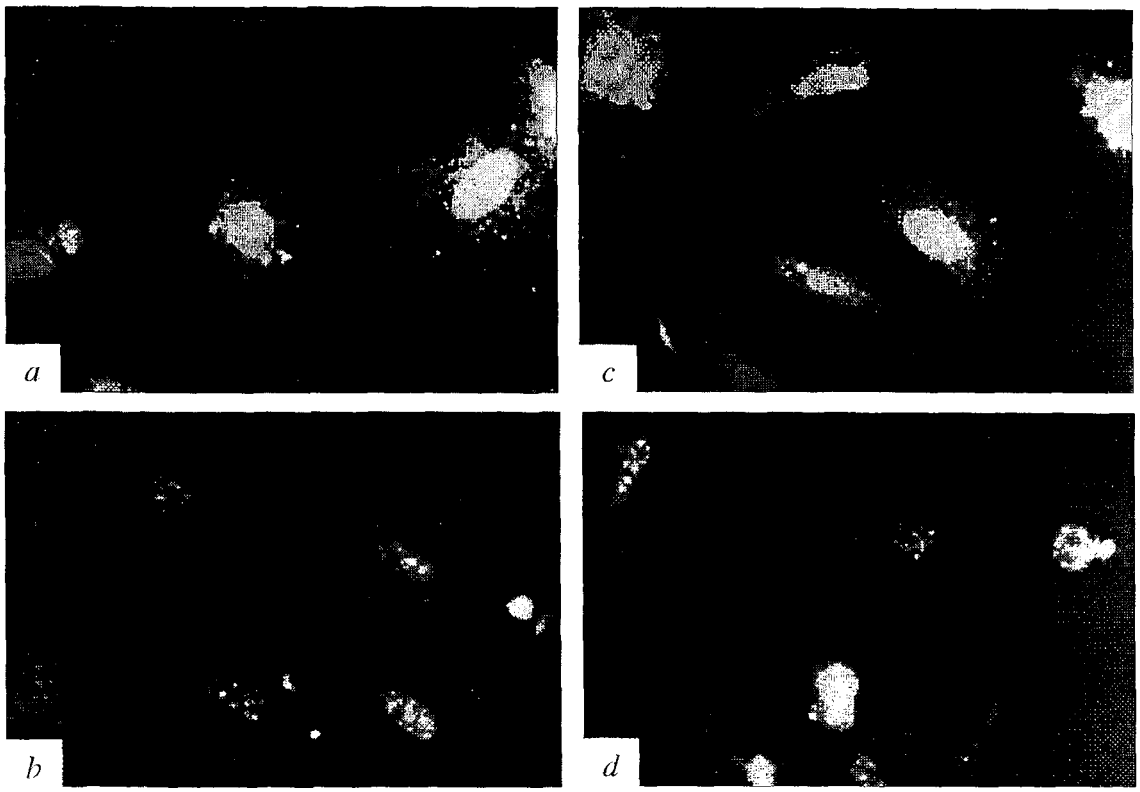

Fig. 2. Visualization(*a*), redistribution (*b*), and prevention of redistribution (*c*) of HSP27.

electric focussing and immunoblotting showed that 2-h incubation of control cells in the presence of CCCP modified the spectrum of HSP27 isoforms; specifically, it induced accumulation of nonphosphorylated a-isoform, a decrease in monophosphorylated b-isoform, and disappearance of di- and triphosphorylated c- and d-isoforms (Fig. 3, *a*). In tolerant EC the ratio of HSP27 isoforms did not change during the ischemia-simulating stress (Fig. 3, *b*). This protective effect cannot be attributed to preserved ATP content in the HS-pretreated cells (Table 1). The time course of intracellular Ca^{2+} during ischemia-simulating metabolic stress was the same in the control and tolerant EC (Table 1). The period of EC protection by HS (12-18 h) coincided with the period of increased HSP27 content (Fig. 4, *a*). Incubation of heated cells with the inhibitor of protein synthesis cycloheximide (50 μM) or HSP production blocker quercetin (30 μM) prevented HS-induced accumulation of HSP27 (Fig. 4, *b*) and abolished the protective effect of HS (Fig. 1, *d* and Fig. 2, *d*).

These data and published reports on the HSP27 effect on polymerization of actin filaments suggest

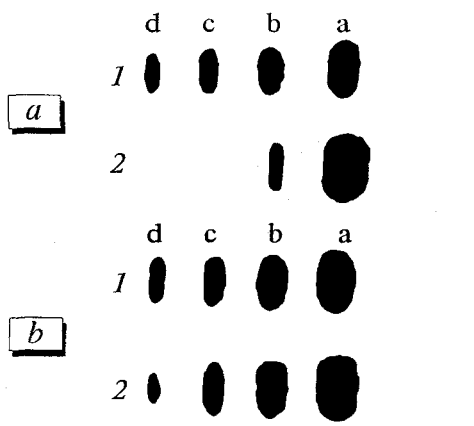

Fig. 3. HSP27 isoforms in ischemia-simulating metabolic stress in control (*a*) and tolerant (*b*) endothelial cells. Isoelectric focussing and immunoblotting before (*1*) and after 2-h ischemia-simulating metabolic stress (*2*).

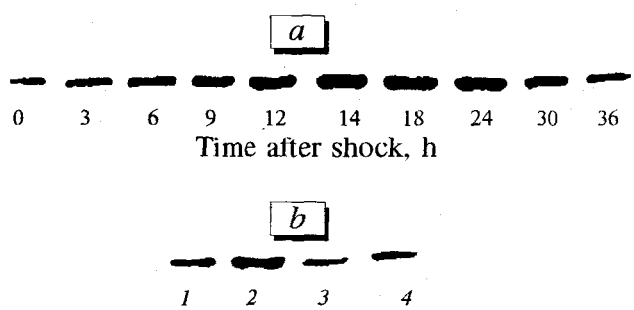

Fig. 4. Heat shock-induced accumulation of HSP27 (*a*) and inhibitory effects of quercetin and cycloheximide (*b*). A blot fragment (isoelectric focussing imaging). *b*) expression of HSP27 in control endothelial cells (*1*), after 18-h recovery (*2*), the same in the presence of 50 μM cycloheximide (*3*) and 30 μM quercetin (*4*).

that HSP27 protects cells from ischemic damage by maintaining the integrity of their actin cytoskeleton. The protective effect of HS is due to accumulation of HSP27 and inhibition of its dephosphorylation upon ATP depletion.

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