Cell Stress & Chaperones (2000) **5** (5), 412–414

Article no. csac. 2000.249

Tissue-level cytoprotection

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Abstract In vitro and ex vivo tissue models provide a useful level of biological organization for cytoprotection studies positioned between cultured cells and intact animals. We have used 2 such models, primary tissue cultures of winter flounder renal secretory epithelium and ex vivo preparations of rat intestinal tissues, the latter to access the microcirculation of exposed mesentery tissues. Herein we discuss studies indicating that differentiated functions are altered in thermotolerant or cytoprotected tissues. These functions include transepithelial transport in renal epithelium and attachment and transmigration of leukocytes across vascular endothelium in response to mediators of inflammation. Evidence pointing to inflammation as a major venue for the heat shock response in vertebrates continues to mount. One such venue is wound healing. Heat shock proteins are induced early in wound responses, and some are released into the extracellular wound fluid where they appear to function as proinflammatory cytokines. However, within responding cells in the wound, heat shock proteins contribute to the acquisition of a state of cytoprotection that protects cells from the hostile environment of the wound, an environment created to destroy pathogens and essentially sterilize the wound. We propose that the cytoprotected state is an anti-inflammatory state that contributes to limiting the inflammatory response; that is, it serves as a brake on inflammation.

INTRODUCTION

Cytoprotection, acquired cellular tolerance of a normally lethal stress induced by sublethal exposure to the same or a different toxicant (cross-tolerance), has been studied mainly using permanent cell lines and heat to induce thermotolerance. Cells in this state are nonproliferative, anti-apoptotic, and anti-inflammatory. Intact vertebrates can be made thermotolerant using a similar protocol involving sublethal heating, a normothermic recovery period, and a challenge with a normally lethal thermal dose to assess protection (White et al 1994). It follows that cytoprotection occurs at the tissue level, the unit of function in multicellular organisms, and may affect processes that only occur in the context of intact tissues. We have studied cytoprotection in 2 tissue models, a renal secretory epithelium established in vitro from winter flounder kidney tubules (Brown et al 1992; Renfro et al 1993) and an ex vivo preparation of rat vascular endothelium. Herein we discuss novel properties of tissue-level cytoprotection.

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Received 31 July 2000; Revised 29 August 2000; Accepted 30 August 2000.

Protection of renal epithelial transport

Secretion of small organic molecules and ions across the renal epithelium requires an intact, differentiated monolayer of epithelial cells complete with tight junctions and apical membrane domains with microvilli. Therefore, protection of net secretory function can only be tested at the tissue level. Dissociation of winter flounder renal tubules yields a population of cells highly enriched for secretory epithelial cells, which reform a functional secretory epithelium when plated on native collagen. After 12 to 14 days incubation at 22°C, the monolayers on collagen pads were mounted in Ussing chambers in which transepithelial electrical characteristics and unidirectional [^{35}S]sulfate fluxes were measured. Sublethal heating and recovery (27°C for 6 hours followed by 1.5 hours at 22°C), that is, stress conditioning, resulted in a 30% increase in sulfate transport rate. Cycloheximide or actinomycin D prevented the enhancing and protective effects of stress conditioning and blocked the induction of heat shock proteins. A challenge severe heat shock (32°C for 1.5 hours followed by 1.5 hours at 22°C) reduced transport by about 30%, essentially to control levels.

Zinc ions can also be used to stress condition the epithelium with similar results. Preincubation of primary epithelium in 100 μM $ZnCl_2$ for 6 hours followed by a 1.5 hours recovery in zinc-free medium enhanced net sulfate flux and protected transport from a severe heat shock. Cycloheximide prevented the induction of heat shock proteins in response to treatment with zinc ions and prevented the acquisition of protection. Induction of cytoprotection by zinc was not specific for sulfate transport since sodium-dependent glucose transport was also protected.

Essentially all hypotheses on the mechanism of cytoprotection have assumed that the protection allows cells to return to near-normal physiological functions by stopping damage to macromolecules and/or facilitating their repair. In our studies it was shown that cytoprotection is characterized by both the presence of stress proteins and increased renal secretory capacity well above control levels. The actual protection of transport is not due to a lack of damage to or repair of transporters but rather to extra capacity, which is inactivated during the challenge stress, but only back to control levels. It is possible that higher amounts of molecular chaperones in the stress-conditioned epithelium allow the assembly of more transporters by a direct chaperoning function. An interesting morphological effect was observed using scanning electron microscopy. Microvilli, in which sulfate transporters are located, disappear after a severe heat treatment, presumably because of the effects on cytoskeletal elements; however, these structures remain after thermal challenge of cytoprotected epithelium.

Inhibition of leukocyte-endothelial cell adhesion in stress-conditioned venules

Vascular endothelium also has functions that require an intact, differentiated monolayer of cells (eg, attachment and transendothelial migration of activated leukocytes from the bloodstream into tissues in the early stages of inflammation). How does stress conditioning affect this tissue-level function? To answer this question, rats were subjected to either heating to 42°C for 15 minutes followed by a 2-day recovery period or to injection IP with stannous chloride (0.15 mg/kg) and exposure for 16 hours. The rats were then prepared for intravital microscopy by exposing mesentery tissue pulled through a midsagittal abdominal incision. The microcirculation of the exposed mesentery tissue was observed using a Nikon UM3 metallurgic microscope adapted for intravital microscopy. Microcirculatory events were recorded using a video camera and videocassette recorder. Stress conditioning rats with either heat or stannous chloride blocks extravasation of neutrophils across venules in response to a proinflammatory stimulus. Since white blood cell flux decreased significantly in response to the proinflammatory peptide formyl-methionyl-leucyl-phenylalanine (FMLP) in both conditioned and placebo animals, we concluded that the initial low-affinity interactions between lymphocytes and endothelium were not blocked. However, firm attachment, measured in a leukocyte-endothelial adhesion assay, was blocked in conditioned animals. Hsp70 was detected by Western blotting of extracts of aortas from heat shocked and stannous chloride–treated rats but not in aortas from placebo rats. Our working hypothesis is that vascular endothelial cells and/or neutrophils are in a cytoprotected state in which they do not respond to signals that would normally up-regulate cellular adhesion molecules involved in the firm attachment of neutrophils to the vascular endothelium.

DISCUSSION

How does the classical heat shock response work in an animal? Our studies indicate that differentiated functions specific to a particular tissue are altered in thermotolerant (cytoprotected) animals, functions such as transepithelial transport in renal epithelium and attachment and transmigration of leukocytes across vascular endothelium in response to mediators of inflammation. One venue of inflammatory responses in vertebrates is wound healing. Inflammation of a wound is essential for proper healing, but prolonged inflammation and excessive destruction of cells in the wound interfere with healing. We propose that heat shock proteins are induced relatively early in wound responses, and cytoprotection begins to develop, a process that requires about 6 to 8 hours. Ian Brown and colleagues have documented the accumulation of Hsp70 mRNA in surgical wounds in rat brain tissue (Brown et al 1989). Cytoprotection would serve as a brake on inflammation, protecting cells from oxidative and heat damage in the inflamed wound and contributing to the throttling down of the inflammatory response, in part by shutdown of signal transduction pathways, as suggested here later. Hightower and White (1981) suggested that inflammation is a major venue for the heat shock response and Barbara Polla suggested that it may serve as a brake on inflammatory responses (Polla 1988). Blood vessels are now returning to center stage in studies of cytoprotection in intact animals and tissues. We say ''returning'' because the studies of Fredric White done 20 years ago (White 1980a; White 1980b) showed that cells associated with the brain microvasculature are among the most stress-responsive cells in explants and in heat shocked rats.

Previous studies have shown that cytoprotected cells are unresponsive to inducers of proliferation and apoptosis. We now add a proinflammatory mediator to this list. Recent studies suggest that a major reason for the unre-

sponsiveness of cytoprotected cells is that products of stress-inducible genes block signal transduction pathways. For example, Sherman and coworkers showed that Hsp70 prevents the activation of JNK and p38 kinases and inhibits heat-induced apoptosis in human tumor cell lines (Gabai et al 1997). The mechanism involves increased rate of inactivation of stress kinase JNK (Volloch et al 2000). Wong and coworkers obtained data suggesting that heat induction of the inhibitory protein I-κB inhibits the activation of the proinflammatory transcription factor NF-κB (Wong et al 1999). Calderwood and colleagues found another anti-inflammatory effect of the heat shock response: Transcription factor Hsf1 acts as a transcriptional repressor of genes encoding several proinflammatory cytokines, including IL1β and TNFα (Xie et al 1999). A transient period of unresponsiveness appears to be an important general characteristic of the cytoprotected state of cell physiology.

ACKNOWLEDGMENTS

Studies of the winter flounder renal epithelium were funded by the Connecticut DEP Long Island Sound Research Fund, the National Institute of Environmental Health Sciences, the National Science Foundation, and the University of Connecticut Research Foundation. M.A.B. was a predoctoral fellow of the University of Connecticut Marine Sciences Institute, and Ms Lauren Barber provided technical assistance. The studies of vascular endothelium were funded by the US Public Health Service, a contract from StressGen Biotechnologies Corp, and the Hartford Hospital Research Fund.

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