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The expression of heat shock protein 70 in the dental pulp following trauma

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Abstract – *Background/Aim*: Traumatic dental injuries vary in severity from mild concussion to avulsion. All organisms respond to stress by inducing the synthesis of a group of proteins known generically as heat shock proteins. The activation of these proteins is an essential cellular mechanism designed to protect against a variety of environmental stresses. It is probable that the production of these proteins is increased in the cells of the traumatized dental pulp, however, this has not as yet been demonstrated. The degree of heat shock proteins expression may be related to the severity of the trauma such that estimating the extent of heat shock proteins expression may provide a practicable way of quantifying these injuries. *Materials and Methods*: Twenty adult male ferrets were divided into four groups. Each maxillary and mandibular right canine was traumatized with the contralateral canines acting as undamaged controls. The trauma was a simulated concussion injury applied using a uniform force. Animals were killed at 24, 48, 72 and 168 h after the injury. The canines were extracted, sectioned and processed for immunohistochemistry using a mouse monoclonal antibody specific for Hsp70. *Results*: There was a statistically significant difference in Hsp70 staining between traumatized and non-traumatized teeth only in the group observed 24 h after the trauma. The expression of heat shock proteins form part of the early pulpal response to trauma.

Traumatic dental injuries vary in severity from mild concussion, which does not loosen the tooth, to avulsion (1). Epidemiologically, the incidence of traumatic injury has increased (2). Experimental work on the response of the dental pulp to trauma has been nominal by the lack of a technique to measure the severity of an injury. Tissues react to stress in many ways. One response is the synthesis of heat shock proteins which stabilize intracellular structure (3, 4). These proteins are critical for cell survival and have several mechanisms of action, which includes regulation of important pro-inflammatory transcription factors (5).

Our hypothesis is that the expression of these proteins in the traumatized dental pulp is related to the magnitude of the trauma. This finding could play an important role as a possible marker to evaluate the extent of trauma in experimental investigations. In this study, we utilized a method in which an uniform and reproducible force could be applied to the tooth. We then measured the expression of one of the heat shock proteins at various times following the injury (6).

Hsp70 is the best known heat shock protein and has been reported in brain (7), heart (8) and the dental pulp (9). These proteins have been reported as present in the pulp during a number of stressful conditions including development (9), formation of reparative dentin (10), cavity preparation (11), following replantation (12) and orthodontic tooth movement (13).

As the expression of these proteins may be related to the severity of the trauma, it is reasonable to anticipate that estimating the extent of heat shock proteins expression may provide a practicable way of comparing injuries.

The objectives of this study were:

1 To determine whether Hsp70 was expressed in the pulps of mechanically traumatized teeth.
2 To investigate whether the degree of expression is related to the degree of pulpal injury.

Materials and methods

Twenty adult male ferrets (Triple Farms, Philadelphia, PA, USA) were used for this study. The protocol was approved by the University of Michigan Committee on Animal Care. Each animal was anesthetized with 30 mg kg^{-1} of ketamine and 10 mg kg^{-1} xylazine both i.m. Each maxillary and mandibular canine on the right side was traumatized leaving the contralateral canines as undamaged controls. A concussion injury was applied by positioning each animal in a plexiglass body holder with the head secured and the mouth propped open and stabilized. A metal sphere weighing 500 mg was released from the top of a 1-m track. At the bottom of the track, the sphere contacted a plunger. The end of the plunger opposite to the sphere was positioned against the canine. Details of this method have been reported in a previous study (6).

Five animals were sacrificed with an overdose of sodium pentobarbital, at each of four time points following the injury: 24, 48, 72 and 168 h. The animals

were then perfused transcardially with 4% depolymerized paraformaldehyde. The canine teeth were removed, demineralized and processed for paraffin embedding.

Table 1. ANOVA analysis for Hsp70

	Sum of square	d.f.	Mean square	*F*-value	*P*-value
Between groups	64577.36	7	9225.338	70.408	<0.0001
Within groups	9433.91	72	131.027		
Total	74011.28	79			

Table 2. Duncan: mean of staining levels for Hsp70

Time/GRP	*N*	Mean*
CONT 24	10	70.49
CONT 48	10	45.90
CONT 72	10	43.25
CONT 168	10	50.93
EXP 24	10	138.98
EXP 48	10	66.45
EXP 72	10	68.37
EXP 168	10	66.31

*Statistically significant.

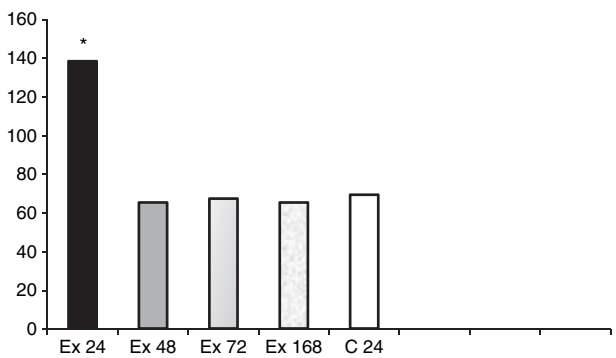

Fig. 1. Mean values of Hsp70 staining levels. *Statistically significant.

Serial sections were cut and stained immunohistochemically using a mouse monoclonal antibody specific for the inducible form of Hsp70 (Sigma, St Louis, MO, USA). Sections of both traumatized and undamaged teeth were examined with the observer unaware of their origin. The observations were made from one field of view of a ×40 objective lens. A digital photomicrograph (Spot RT Diagnostic Instruments Inc., Sterling Heights, MI, USA) was taken under standardized conditions of exposure and illumination of this area as was a comparable area of the pulp from the control teeth. Each image was viewed in IMAGE J software (NIH.GOV; National Institutes of Health, Bethesda, MD, USA). With the image reversed to appear as a negative, the mean grey level of each image was determined by the densitometry feature of IMAGE J. Reversing the image results in more heavily stained tissue, giving a higher number for the mean grey value than lightly stained tissue. At the time of measurement, the observer was unaware whether sections were from traumatized teeth or controls. Technique controls were included in which the specific antibody was replaced with non-immune serum. No staining above background levels was seen in these sections. The numerical data were analyzed with ANOVA as followed by Duncan's *post hoc* test, $P < 0.05$ was accepted as significant.

Results

The numerical data are shown in Tables 1 and 2 and Fig. 1 and the sample micrographs are shown as Figs 2 and 3. There is a statistically significant difference in the density of staining for Hsp70 between the control and experimental groups 24 h after the injury ($P < 0.001$), but not at the later time points.

Discussion

The increased expression of Hsp70 at 24 h but not 48 h after injury suggests that this increase is a component of the tissues early response to trauma and that this response is reversed within another 24 h. In this investigation as well as in a previous study (6), an increase in the number and size of vessels starting at 24 h following

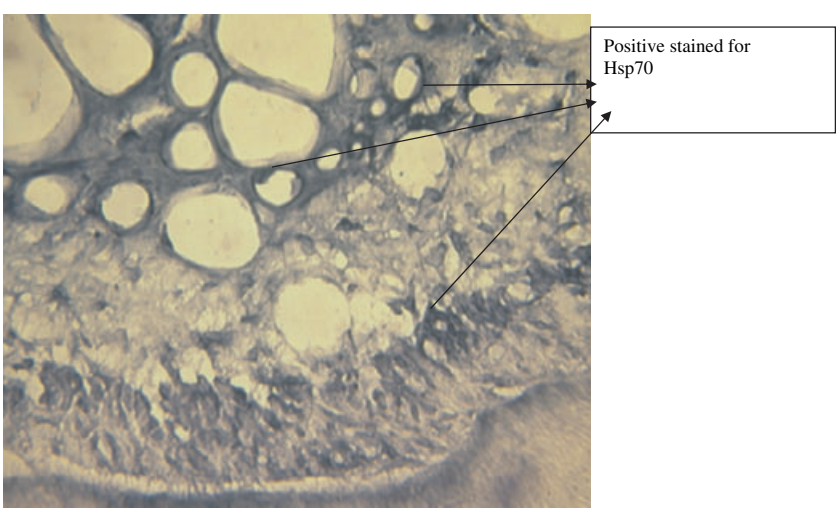

Fig. 2. Twenty-four hour experimental. Immunolocalization of Hsp70 with monoclonal antibody.

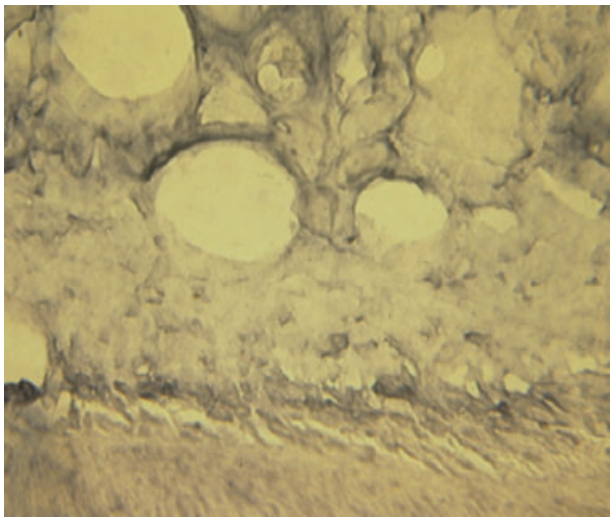

Fig. 3. Control 24 h.

injury was noted, and the vessels returned to normal when the inflammatory process subsided.

Thus, it is unsurprising that the levels of Hsp70 return to normal values when there is no further stress as the blow used in this study was relatively mild. The clinical findings following similar trauma are usually a sensitivity to percussion that can be treated with occlusal adjustment (14). Only 3% of these cases result in pulpal necrosis and neither the periodontal ligament nor the pulpal neurovascular supply are thought to be significantly compromised (15).

Considering that concussion and subluxation injuries have few healing complications (16), this injury was selected to function as baseline for future studies with more severe injuries. In this preliminary study, we demonstrated that an expression of the inducible form of Hsp70 occurred in all the traumatized considerably higher at 24-h postinjury than the other following time periods.

This study demonstrated that increased Hsp70 expression occurs in the dental pulp even after minor trauma. The intensity of its expression is highest 24 h after injury and declines to normal a day later. This is in agreement with studies on other tissues (17–19).

Based on the results of this study, it can be suggested that staining for heat shock proteins may provide a method of measuring the degree of trauma. Further studies using more severe trauma are needed to confirm this.

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