The Effects of High Thoracic Epidural Anesthesia on Sympathetic Activity and Apoptosis in Experimentally Induced Congestive Heart Failure

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Objective: **To evaluate the effect of high thoracic epidural analgesia (HTEA) in congestive heart failure (CHF).**

Design: **Rat model of CHF.**

Setting: **Harbin Medical University, Harbin, Heilongjiang, China.**

Participants: **One hundred thirty-five rats.**

Interventions: **HTEA involved 5 times daily injections of 0.1% lidocaine at the T3-T4 level.**

Measurements and Main Results: **The authors examined myocardial norepinephrine (NE), angiotensin II (Ang II), endothelin-1 (ET1), and tumor necrosis factor-α (TNF-α) concentrations 2, 4, and 6 weeks after the start of HTEA. They also examined histologic changes in heart tissue and myocardial expression of apoptosis-inducing factor (AIF) and poly (ADP-ribose) polymerase (PARP). Sham rats were used as a control. In the time course, myocardial NE, Ang II, ET1, and TNF-α concentrations were significantly higher in the CHF group compared with the HTEA and sham groups ($p < 0.05$). Similarly, PARP and AIF protein expression levels were significantly higher in the CHF group compared with the HTEA and sham groups ($p < 0.05$). Microscopy revealed pronounced damage to myocardial cell structures in the CHF group; this damage clearly was reduced in the HTEA group. In addition, cardiac function evaluation indicated treatment with HTEA resulted in similar heart function as animals that did not have surgically induced CHF.**

Conclusions: **The findings suggest that HTEA induces changes in sympathetic nervous system, renin-angiotensin system, endothelial, and inflammatory process activity involved in CHF.**

KEY WORDS: apoptosis, congestive heart failure, cytokines, high thoracic epidural analgesia, neurohumoral factors

CONGESTIVE HEART FAILURE (CHF) due to cardiovascular disease is extremely common and is associated with significant morbidity, mortality, and financial costs,[1] and the prevalence of CHF is expected to continue increasing as the population ages.[2] Although current treatments for CHF can be effective,[2] there is a clear need for continued research and development into alternative therapeutic options.[3]

One potential option for the treatment of CHF is high thoracic epidural analgesia (HTEA). There is evidence to suggest that HTEA improves myocardial blood flow and left ventricular function in patients with ischemic heart disease[4–6] and relieves refractory angina pectoralis.[7–10] There also have been several reports published within the last 5 years suggesting the HTEA may improve cardiac function in patients and animals with CHF. Specifically, Wu et al[11] reported that HTEA improved hemodynamics and the clinical condition of a patient with end-stage congestive heart failure who had not responded to other medical treatments. In another clinical study, Wu et al[12] found that cardiac function was significantly improved after HTEA in patients with CHF compared with control patients who did not receive HTEA. Further, Chen et al[13] found that HTEA improved ventricular remodeling and cardiac function in rats with experimentally induced heart failure.

The mechanisms through which HTEA might improve cardiac function in CHF are unknown. During the onset and development of CHF, systolic dysfunction leads to overactivation of the neuroendocrine system,[14] in particular the sympathetic, endothelial, and renin-angiotensin (RAS) systems.[15–17] Activation of these systems can lead to excessive release of catecholamines,[18] tumor necrosis factor-α (TNF-α),[19] endothelin-1 (ET-1)[20] and angiotensin II (Ang II),[21] which in turn may directly or indirectly cause inflammation, oxidative stress, myocardial cell injury and apoptosis, and ultimately ventricular remodeling.[22] To further investigate the effect of HTEA on CHF, the authors used a rat model of CHF and examined the impact of HTEA on myocardial concentrations of norepinephrine (NE), Ang II, ET1, and TNF-α. This study also examined histologic changes in heart tissue as well as myocardial expression of apoptosis-inducing factor (AIF) and poly (ADP-ribose) polymerase (PARP), an important mediator of DNA repair after ischemic injury.[23]

MATERIALS AND METHODS

All animal experimentation was performed in compliance with the guidelines of the Animal Ethics Committee of the Harbin Medical University (Harbin, Heilongjiang, China).

A total of 135 healthy male adult Wister rats (First Clinical Medical College of Harbin Medical University, Harbin, China) weighing from 200 to 300 g (mean: 260 ± 58 g) were used. The rats were divided into the following 3 experimental groups: CHF (n = 45), HTEA (n = 45), and sham (n = 45). Rats in each group were further divided into groups for sampling at 2-, 4-, and 6-week intervals. Animals were randomized using a random number table.

The authors used an established model of CHF.[24,25] Briefly, rats were anesthetized with 1% pentobarbital sodium (40 mg/kg) and placed in a supine position before being connected to a MAC1200 Resting Electrocardiogram (ECG) System (GE, Milwaukee, WI). A thoracic incision was made to the left of the midline, and the heart was elevated. The left anterior descending coronary artery was located and the main trunk ligated with 3/0 silk suture. (All procedures except for ligation were performed for rats in the sham group.) This ligation produces ischemic cardiomyopathy. The heart was placed back in the thoracic cavity, and the thoracic wall was closed. ST-segment elevation in the anterior precordial leads was taken to indicate the successful induction

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of acute myocardial infarction. After 6 weeks, hemodynamics were measured using a RM-600 4-channel physiologic recorder (Nihon Kohden, Tokyo, Japan). Stroke volume, minute volume, and cardiac index (CI) were calculated using Kubicek's formula.[26] Heart failure was considered to be established if CI was less than 40% of normal.

After surgery, the animals stayed at 1 mouse per cage to avoid attacks from other animals. The authors also designed a wood clip-like Elizabethan collar similar to that used for a cat or dog, to block the ability of the animal to bite the epidural catheter. The staff of the animal center of The First Clinical Medical College of Harbin Medical University cared for the animals. Animals had free access to water, and they were fed regularly. All animals were cared for under the same conditions, including the diet and day-night cycle. Motor functions were not tested

Rats with established CHF were anesthetized with 1% pentobarbital sodium (40 mg/kg) and placed in a supine position on an operating table. Hair around the T4 and T5 spinous processes was shaved, and a skin incision was made after sterilization. Subcutaneous tissue, the supraspinous ligament, interspinous ligament, and ligamentum flavum were bluntly dissected between T4 and T5. The dura mater gently was punctured with a needle to expose the epidural space. An epidural catheter with a front-end diameter of 1 mm was inserted into the epidural space and advanced cephalad approximately 2 to 3 mm to the T3-T4 level. The epidural catheter then was fixed and the skin was closed. Red ink was injected into the epidural catheter to ensure blockade at the T1-T5 level.

After placement of the epidural catheter, 50 μL of 0.1% lidocaine (Shanghai Fuxing Zhaohui Pharmaceutical Co., Ltd., Shanghai, China) was injected 5 times daily, every 2 hours from 8:00 a.m. to 4:00 p.m., and continued to 2, 4, 6 weeks according to the assessment times of the rats. Each injection was completed within 15 seconds. Successful epidural block was indicated by a skin temperature increase of 0.5°C.

Rats were sacrificed 2, 4, and 6 weeks after the start of HTEA. The following variables were assessed: myocardial concentrations of NE, Ang II, ET1, and TNF-α, myocardial tissue protein expression of PARP and AIF, and myocardial tissue morphology. Infarcted regions of left ventricular anterior wall were collected for these assays.

Approximately 30 to 50 mg of tissue were obtained from the anterior wall of the left ventricle and frozen at −80°C for 2 to 3 minutes. Samples then were placed in 100 μL dehydrated alcohol and fixed for 1 minute. 900 μL of normal saline then were added and the samples were homogenized. Homogenates were stored at −40°C until assay.

Concentrations of NE, Ang II, ET1, and TNF-α were assessed using commercial radioimmunoassay kits (NE, Ang II: Beijing Chemclin Biotech Co., Ltd, Beijing, China; ET1, TNF-α: Beijing Beimian Dongya Institute of Biological Technology, Beijing, China).

Myocardial protein expression levels of PARP and AIF were determined using immunohistochemistry kits. For PARP and AIF antigens detection, the myocardial sections were incubated with rabbit anti-rat PARP and rabbit anti-rat AIF (Santa Cruz, CA) and were detected by DAB staining kit (Santa Cruz, CA) according to the manufacturer's protocol. After extensive washing, the sections were mounted and observed through a light microscope (×400). Image analysis was performed using image analysis software Image-Pro Plus 6 (Media Cybernetics, Silver Spring, MD). Eight visual fields were selected randomly at high magnification and the percentage area of AIF and PARP expression in each visual field was calculated. The mean values were taken for statistical analysis.

Heart tissue was processed and fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Three μm slices of myocardial tissue were examined using a light microscope (Olympus BX60; Olympus, Tokyo, Japan) to assess the histopathologic features in each group. Samples obtained at week 6 also were examined by electron microscopy. Pathologists who examined the samples were blinded to group assignments.

Continuous variables are presented as the mean ± standard deviation. The effects of group and time on concentrations of NE, Ang II, ET1, and TNF-α and protein expression levels of PARP and AIF were determined using 2-way analysis of variance. When a significant between-groups difference was apparent, multiple comparisons of means were performed using the Student-Newman-Keuls test. All statistical assessments were 2-sided and considered to be statistically significant when $p < 0.05$. Statistical analyses were performed using SPSS 15.0 statistical software (SPSS Inc, Chicago, IL).

RESULTS

A total of 31 rats died during the surgery that established CHF; 6, 10, and 15 rats in the sham (mock surgery with no ligation), CHF (CHF induced but animals not treated with HTEA), and HTEA groups (CHF induced and treated with HTEA), respectively. The success rate for development of CHF was 23%. In general, HTEA reduced the heart rate about 10 to 20 beats/min of the rats. At 6 weeks, 14/15, 12/15, and 9/15 animals were alive for the sham, CHF, and HTEA groups, respectively.

There were significant effects of group and time on myocardial concentrations of NE, Ang II, ET1, and TNF-α (all $p < 0.001$). Concentrations of NE, Ang II, ET1, and TNF-α were significantly lower in the HTEA and sham groups compared with the CHF group over time ($P < 0.05$). In addition, significant differences between the sham and HTEA groups were found. Concentrations of NE, Ang II, ET1, and TNF-α tended to significantly decrease with time in all 3 groups ($p < 0.05$) (Fig 1).

There were significant effects of group and time on myocardial protein expression of PARP and AIF ($p < 0.001$, Fig 2, Fig 3). Myocardial expression levels of both PARP and AIF were significantly lower in the HTEA and sham groups compared with the CHF group during the time course ($p < 0.05$). In addition, significant differences between the sham and HTEA groups were found. Myocardial expression levels of PARP and AIF significantly decreased with time in all 3 groups ($p < 0.05$).

Light microscopy revealed variable structural cell damage, loss of membrane structure, inflammatory cell infiltration, and fibrosis of myocardial cells in samples from rats in the CHF group at weeks 2, 4, and 6 (Fig 4A). In contrast, structural cell damage appeared markedly reduced in samples from rats in the HTEA group compared with the CHF group (Fig 4A).

Transmission electron microscopy revealed completely dissolved myofilaments within the myocardial cells, intercalated disc rupture, sarcomere structure disappearance, nuclear pyknosis and irregular morphology, mass-like spread of high-density chromatin in the nucleus, perinuclear mitochondria derangement and breakage, decreased matrix density, and abnormal sarcomere contraction bands in samples from rats in the CHF group (Fig 4B). In contrast, samples from rats in the HTEA group revealed myocardial cells with intact and slightly thickened membranes, clear sarcomere bands, maintained