Total Sleep Deprivation Augments Balloon Angioplasty-Induced Neointimal Hyperplasia in the Sprague-Dawley Rat

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ABSTRACT

Objective/Background: Sleep deprivation has been shown to be associated with an increase in inflammation that also involved in the development of neointimal hyperplasia (or restenosis). The purpose of this study was to investigate whether total sleep deprivation (TSD) will worsen neointimal formation by balloon injury.

Methods: Sixteen rats were randomly allocated into four groups: Group C, balloon angioplasty alone; Group A, TSD prior to angioplasty; Group B, angioplasty before TSD; Group AB, TSD before and after angioplasty. TSD was induced by the disc-on-water method, and balloon angioplasty was performed in rat carotid artery. The histopathological analysis and cytokines assay were applied to evaluate the effects of TSD in this study.

Results: TSD significantly increased the ratio of post-injury neointima-to-media area in Group A ($p < 0.05$), Group B ($p < 0.05$) and Group AB ($p < 0.01$) as compared with those of Group C. Additionally, all groups of TSD administration also decreased the serum level of interleukin-10 (IL-10) at day 2 and day 3 after angioplasty injury ($p < 0.05$).

Conclusions: Our preliminary finding suggested that perioperative TSD can significantly augment neointimal hyperplasia of carotid artery in rats, which may be partially caused by TSD-induced effect in suppressing the serum level of anti-inflammatory cytokine, IL-10.

Keywords: balloon angioplasty, inflammation, total sleep deprivation, neointimal hyperplasia

1. Introduction

Sleep deprivation or insomnia, an extremely common ailment in modern society, may affect numerous neurobehavioral and physiological functions such as memory, cognitive ability, hormone secretion, glucose metabolism, and immune function, etc. [1-3]. Numerous studies have implicated associations between sleep deprivation and inflammatory responses, although the pathophysiological mechanisms underlying this association remain unclear [4-6]. Notably, systemic inflammation has been demonstrated to play a critical role in the progression of many chronic diseases, and it also associated with increased risk of various diseased conditions such as cardiovascular diseases, tumorigenesis and autoimmune diseases, etc. [7-9].

Balloon angioplasty or percutaneous transluminal coronary angioplasty (PTCA) is a routine medical procedure used for expanding narrowed coronary arteries and then restore the blood supply to the infarcted regions of heart tissue. However, about 30~50% of patients received balloon angioplasty will suffer from a surgical complication—neointimal hyperplasia (or restenosis) within 6 months, which has become the major limitation for clinical application of PTCA. Restenosis is a complex and multifactorial process involving arterial remodeling and neointimal hyperplasia, which might be caused by endothelial disruption, cell proliferation and migration of vascular smooth muscle cells (VSMCs) [10]. The pivotal role of inflammation in the development of restenosis has

been indicated in several research articles [11-13], and several evidences of anti-inflammatory therapy on the preventing neointimal formation have been demonstrated to effectively reduce angioplasty restenosis [14-16].

Although numbers of researches have indicated the correlations between sleep deprivation and inflammatory response, the inflammatory effect of sleep deprivation on balloon angioplasty-induced neointimal hyperplasia is still unclear. Thereby, rats were induced total sleep deprivation (TSD) by using disc-on-water method to evaluate whether neointimal formation will be affected by TSD before/after balloon angioplasty.

2. Methods

2.1. Experimental animals

Male Sprague-Dawley rats (5-6 weeks old, 200-300 g) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan) and housed in stainless steel cages with 12 h light/dark cycles with free access to food and water. The experimental protocol for this study was approved by grant 97-85-N from the Institutional Animal Care and Use Committee (IACUC) at the China Medical University (Taichung, Taiwan). The institutional animal ethical guidelines of US National Institutes of Health guidelines and the China Medical University were followed for animal care.

Sixteen rats were randomly allocated into four groups: Group C, balloon angioplasty alone without sleep deprivation; Group A, balloon angioplasty after 24 h sleep deprivation; Group B, balloon angioplasty before 24 h sleep deprivation; Group AB, 24 h sleep deprivation before and after balloon angioplasty (Fig. 1). Rats were anesthetized by intramuscular injection of 20mg/kg Zoletil 50® with 10mg/kg Rompun®. For monitoring sleep status in the experimental rats, four electroencephalography (EEG) electrodes were implanted into the right lateral frontoparietal, right and left medial (bregma) parietal, and left lateral lambda-parietal cortex. One additional pair of nickel-chromium fine-wire electrodes was implanted in the dorsal neck muscle for electromyogram (EMG) recording. The electrodes were soldered to a connector, which was fixed to the animal cranium with

acrylic dental cement. After the implantation surgery, rats given penicillin and diclofenac and maintained on 12 h light/dark cycles at 25°C for two days for healing.

2.2. Total sleep deprivation

A 24 h period of TSD was induced by the dish-over-water method using a modified Rechtschaffen apparatus (Fig. 2). Before TSD, the rats were raised in the disc-water cages for five days to adapt to the environment, and the functioning apparatus was placed among other four plastic chambers with rats during the TSD experiment for preventing the isolation-induced stress effects in rats. At beginning of study, the implanted electrodes were connected to an EEG monitoring system (MP150; Biopac System Inc., USA) to obtain EEG data by acquisition software (Acqknowledge ver. 3.7.3; Biopac System Inc., USA). The sleep state was identified by the root square of the EEG-theta wave. When an increase in theta waves was detected, the computer started the motor beneath the disc to rotate the disc counterclockwise at a moderate speed of 3.5 rpm. At this time, the rat was awakened by disc rotation which forced the animal to walk in the opposite direction and avoid falling into the water tray, and disc rotation would be stopped as the monitoring system detected a decrease in theta wave activity. This whole procedure continued for 24 hours resulting in TSD. A sampler test was done to compare the efficacy of identifying EEG sleep state, showing that have 90 percent similarity between manual (visual) and

computer automatic interpretations.

2.3. Balloon angioplasty of rat's carotid artery

Balloon angioplasty was performed as our previous report [17]. In brief, rats were anesthetized by intraperitoneal injection of 3.6% (w/v) chloral hydrate (1 ml/100 g). Subsequently, the balloon catheter (2F Fogarty) (Becton-Dickinson, Franklin Lakes, NJ, USA) was introduced through the right external carotid artery into the aorta, and the balloon was inflated at 1.3 kg/cm^2 using an inflation device. An inflated balloon was pushed and pulled through the lumen three times to damage the vessel. After completing surgery, the catheter was removed, the external carotid was ligated and the wound was closed. These rats were maintained on 12 h light/dark cycles at 25°C for recovery.

2.4. Histopathological analysis

Rats were sacrificed by use of overdose of desflurane, and rats were perfused transcardially with normal saline solution. Both the right and left carotid arteries (approximately 2 cm in length) were isolated, soaked with 10% formalin at 4°C overnight, and embedded in Parafilm block. Embedded vessel tissues were cut into 10 μm-thick slices, and then, slices were stained with hematoxylin (Merck, Argentina, USA) and eosin Y (Merck). Cross-sectional segments from both left and right carotid arteries were further

analyzed by using a light microscope (Nikon, Optophot, Mississauga, ON) under the 40× magnifications. Morphometric analysis was carried out using AlphaEaseFC image analyzer software to measure the surface area (mm^2). The manifestation of vessel restenosis was presented as the ratio of neointima-to-media area.

2.5. Measurement of serum cytokines

Blood samples were drawn from the tail vein of each rat before injury (day 0), and 1, 2, 3 and 14 days after balloon angioplasty. Serum was separated by centrifugation at 3000 rpm for 15 min and then stored at -80°C until assayed. Serum levels of interleukin-6 (IL-6), IL-10 and interferon-gamma (INF-γ) were determined by enzyme-linked immunosorbent assay (ELISA) using a rat IL-6, IL-10 and INF-γ ELISA kit (Bender MedSystems, Austria). The sample test was run in batch and the coefficient of variation of ELISA kits for the same biomarker was less than 5%.

2.6. Statistics

All values are expressed as mean ± standard deviation (SD). Data were compared with one-way analysis of variance (ANOVA) and Bonferroni post-hoc tests to evaluate differences among multiple groups. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Morphometric analysis

Our results revealed that all groups of TSD administration will significantly worsen the severity of neointimal formation no matter before/or after balloon angioplasty (Fig. 3A). The severity of neointimal formation was presented as the ratio of neointima-to-media area (N/M ratio), and higher ratio indicated more intravascular stenosis and neointimal hyperplasia. The post-injury N/M ratio in Group A (1.43 ± 0.35), Group B (1.48 ± 0.30), and Group AB (1.87 ± 0.29) were significantly higher ($p < 0.001$) than those of Group C (0.67 ± 0.23) (Fig. 3B). There was no obvious difference between the Group A and Group B, but the N/M ratio of Group AB was markedly enhanced as compared to those in the Group A or Group B ($p < 0.05$).

3.2. Serum level of cytokines

The experiment data showed that TDS administration did not significantly influence the plasma level of IL-6 and INF-γ at all points in time as compared to those of Group C (Fig. 4A and 4C). Otherwise, the serum level of IL-10 was markedly altered by TSD after balloon angioplasty (Fig. 4B). In the present study, our results found that the level of serum IL-10 of Group C was gradually increase in a time-dependent manner until 3 days post-angioplasty and decreased plasma level at 14th day after angioplasty. In contrast to

the results of Group C, the serum level of IL-10 in all groups of TSD administration was depressed at 2^{th} and 3^{th} day ($p < 0.05$) after balloon injury. However, there were no statistical differences in serum level of IL-10 among all groups of TSD administration at all points in time.

4. Discussion

To our knowledge, our studies demonstrate for the first time that TSD is a risk factor to contribute and worsen the development of neointimal hyperplasia in a rat model of balloon angioplasty. Our results suggested that this adverse effect of TSD on enhancing neointimal proliferation might be partially caused by the suppression of anti-inflammatory cytokine, IL-10.

It has been demonstrated that balloon-angioplasty, particularly with stent implantation, triggered inflammatory reactions leading to the development of intimal hyperplasia. Atrial specimens from human restenotic lesions revealed an increased number of inflammatory cells and monocyte chemoattractant protein-1 (MCP-1) in restenotic lesions [18-20]. Farb *et al.* noted that coronary stenting induces increased arterial inflammation that is associated with increased neointimal growth [18]. Similar findings also indicated that stent implantation is associated with an increase in an acute-phase protein, C-reactive protein (CRP), which significantly higher and more prolonged in patients with restenosis compared to patients without restenosis [21, 22]. Thereby, a number of anti-inflammatory agents have been used as for clinical trial to evaluate the efficiency of preventing neointimal hyperplasia. The REGRESS study (Regression Growth Evaluation Statin Study) suggested that pravastatin treatment reduced 2-year clinical and angiographic restenosis [14]. Non-specific anti-inflammatory agents such as prednizone in the prevention of

restenosis was investigated in the IMPRESS study (Immunosuppressive Therapy for the Prevention of Restenosis after Coronary Artery Stent Implantation), indicating six-month restenosis rate and lumen loss were lower in patients treated with prednisone [16]. Clinical trials of drug-eluting stent coated with paclitaxel, an agent has anti-inflammatory and anti-proliferative properties, have been demonstrated to reduce coronary restenosis [23, 24]. Interestingly, it has been reported that activated inflammatory cells increased local temperature of atherosclerotic plaque [25], which is an independent predictor of clinical outcome in patients undergoing a percutaneous coronary intervention, showing the effect of pre-existing inflammation in the development of restenosis [26].

There are several cytokines, such as IL-1, IL-6, IL-10 and tumor necrosis factor-α (TNF-α), being studied extensively with respect to sleep-wake behavior [27-30]. It was hypothesized that IL-6 is a mediator of sleep because its circadian patterns reflect the homeostatic drive of sleep [31]. Frey *et al*. indicated that 40 h of TSD in healthy young adults can markedly increase serum level of IL-1 but decrease IL-6 level [6]. Hu et al. mentioned that 36 h sleep deprived animals there was a marked increase in cytokines levels (TNFα, IL-1 and IL-6) proposed to regulate sleep behavior in mice [32]. However, IL-6 levels were elevated in the 4 h sleep condition over the 8 h sleep condition [33]. In our study, 24 h TSD did not influence serum level of IL-6 after balloon angioplasty (Fig. 4A). Similarly, at the injured lesion after angioplasty activate and recruit leukocytes, monocytes,

and macrophages from the circulating blood and adventitia, which triggered local inflammation and elicits systemic immune response mediated by inflammatory cytokines, such as IL-1, IL-6, IL-10 and TNF-α [34]. It has been reported that in patients with multi-vessel disease the serum level of IL-6 and IL-10 during hospitalization was significantly higher than in patients with single-vessel disease [35].

IL-10 is an anti-inflammatory cytokine with pleiotropic effects in immunoregulatory functions whose actions influence synthesis of pro-inflammatory cytokines [36] and activities of many types of cells in the immune system. Numerous studies reported that it functions as a potent inhibitor of monocytes [37] as well as decreasing cell adhesion molecules [38], monocyte chemoattractant protein [39], and cell proliferation of vascular smooth muscle cells [40]. In angioplasty lesions, monocytes infiltration has been indicated to correlates with subsequent intimal overgrowth [41]. Thereby, IL-10 has potential effect to decrease the degree of monocytes infiltration or activation and further prevent the development of neoinitmal formation. Feldman *et al.* also proved that increasing plasma level of IL-10 by exogenous supply can provide beneficial effect to markedly decrease angioplasty or stent implantation-caused neointimal proliferation in hypercholesterolemic rabbits [42]. On the other hand, there was increased IL-10 levels achieved by the administration of a recombinant protein or by using a gene delivery system inhibits vascular neointimal proliferation after balloon injury and transplant-related arteriosclerosis [43]. In

the present study, our data revealed that all of TSD administration will significantly inhibit serum level of IL-10 at day 3 after balloon angioplasty as compared with control group (Group C) at the same point in time (Fig. 4B), and this effect of TSD might lead to relatively higher inflammatory response within the angioplasty lesions and worsen neointimal proliferation. Similarly, the ratio of neointima-to-media area was markedly increased in all groups of TSD administration (Fig. 3).

In conclusion, our preliminary finding showed that all groups of TSD can augment balloon angioplasty-stimulated neointimal proliferation in rats, and this effect might be partially caused by suppressing the expression level of serum IL-10. Our model provided an experimental basis on deleterious effects of TSD on post-angioplasty restenosis, and this finding has great implications on clinical care as the patients will receive balloon angioplasty intervention.

Disclosures/conflict of interest

The authors on this project have no disclosures regarding conflicts of interest.

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Figure Legends

Fig. 1. Schematic diagram of the study design. Group C, balloon angioplasty alone without sleep deprivation; Group A, balloon angioplasty after 24 h sleep deprivation; Group B, balloon angioplasty before 24 h sleep deprivation; Group AB, 24 h sleep deprivation before and after balloon angioplasty.

Fig. 2. The overall appearance of the components of TSD inducing apparatus used in the present study. The apparatus consisted of two rectangular clear plastic chambers measuring 60 cm (length) × 20 cm (width) × 60 cm (height), and the two chambers were placed side by side. Each chamber housed a rat, and food and water were adequately provided in the apparatus. A large disc, a programed rotating platform under both chambers, was used to interrupt sleep of rat, and a full-size tray filled with water 2-3 cm in depth was placed beneath the disc.

Fig. 3. Histopathological examination of the rat's carotid arteries. The right carotid arteries harvested from sham control, Group C, Group A, Group B and Group AB at end of the present study, and the slides of vessel tissues were stained to examine the severity of neointimal formation by light microscope at 40-fold magnification (A). Arrow indicates the neointimal layer from the internal elastic fiber. The manifestation of vessel restenosis

was presented as the ratio of neointima-to-media area (B). Histograms of all values are expressed as the mean ± SD from three independent experiments. ** indicates $p < 0.01$ as compared with control group (Group C). ‡ indicates $p < 0.01$ as compared with Group A. # indicates $p < 0.05$ as compared with Group B.

Fig. 4. Change of the level of serum cytokines during the experiment. Blood samples were drawn from the tail vein of each rat before injury (day 0), and 1, 2, 3 and 14 days after balloon angioplasty for detecting serum level of IL-6 (A), IL-10 (B) and IFN-γ (C). Histograms of all values are expressed as the mean ± SD from three independent experiments. * indicates $p < 0.05$ as compared with control group (Group C) at same point in time.