Spinal Cord Injury Induced Arrest in Estrous Cycle of Rats Is Ameliorated by S-nitrosoglutathione: Novel Therapeutic Agent to Treat Amenorrhea

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ABSTRACT

Introduction. Amenorrhea following spinal cord injury (SCI) has been well documented. There has been little research on the underlying molecular mechanisms and therapeutics.

Aim. The purpose of the present study was to investigate the effect of GSNO in ameliorating SCI-induced amenorrhea through affecting the expression of CX43, NFkB, and ERβ protein.

Methods. SCI was induced in female SD rats at the T9-T10 level. Estrous stage was determined by vaginal smear. GSNO (50 μg/kg body weight) was gavage fed daily. Animals were sacrificed on day 7 and 14 post SCI. Ovaries were fixed for histological and biochemical studies. Expression levels of ERβ, CX-43, and NFkB were analyzed by Western blot and immunofluorescence.

Main Outcome Measures. GSNO hastens resumption of the estrous cycle following SCI-induced transient arrest.

Results. Resumption of estrous cycle was hastened by GSNO. Atretic and degenerating follicles seen in the ovary of SCI rats on day 14 post-SCI were decreased in GSNO treated animals. The increased CX43 expression observed with SCI ovary was decreased by GSNO. ERβ expression decreased significantly on day 7 and 14 post-SCI and was restored with GSNO treatment. Following SCI, NFkB expression was increased in the ovarian follicles and the expression was reduced with GSNO administration. The number of terminal deoxynucleotidyl transferase-mediated biotinylated uridine triphosphate (UTP) nick end labeling positive follicular and luteal cells was increased after SCI. GSNO-treated animals had significantly fewer apoptotic cells in the ovary.

Conclusion. SCI-induced amenorrhea is accompanied by an increase in CX43 expression and a decrease in ERβ expression. SCI animals treated with GSNO resumed the estrous cycle significantly earlier. These results indicate a potential therapeutic value for GSNO in treating amenorrhea among SCI patients. **Shunmugavel A, Khan M, Chou PC, and Singh I. Spinal cord injury induced arrest in estrous cycle of rats is ameliorated by S-nitrosoglutathione: Novel therapeutic agent to treat amenorrhea. J Sex Med 2012;9:148–158.**

Key Words. Spinal Cord Injury; Amenorrhea; Sexual Function and Estrous Cycle

Introduction

Regaining sexual function is among the highest priorities of spinal cord injury (SCI)-induced paraplegics [1]. In 2009, 262,000 people were diagnosed with SCI (http://www.miamiproject.miami.edu/Document.Doc?id=197). Since most SCI patients are men, research has focused on their recovery or on issues common to both males and females. Unfortunately, issues particular to female patients have not been investigated to the same degree [2]. However, each year, more than 3,000 women of childbearing age in the United States are afflicted with SCI [3]. In addition, 59% of SCI patients of either sex manifest at least one form of sexual dysfunction [4]. Hence, assessment and management of sexual dysfunction need more research on the pathophysiology and optimal treatment of sexual dysfunction associated with chronic illness like SCI [5,6].

The spinal nerves (T10-L2) [7] and the abdominal branches of the vagus nerve [8] enervate the ovaries. However, anterograde signaling occurs only through the spinal cord [9]. Hence, interruptions in signaling due to SCI alter ovarian physiology through inflammation and apoptosis. Activated nuclear NFkB is often found at the sites of inflammation in the central nervous system [10]. NF-kB is a ubiquitous heterodimeric transcription factor that is sequestered in the cytoplasm by the inhibitor of kappa B (IkB) proteins. Degradation of IkB allows translocation of NFkB into the nucleus and subsequent activation of its target genes.

Amenorrhea may either stem from normal physiological conditions like pregnancy, menopause, and lactation, or develops as a consequence of pathological conditions like SCI. Sleep disturbances (43%), night sweats (40%), decreased sex drive (38%), and amenorrhea (60%) are common consequences of SCI [11]. This interruption in the menstrual cycle is commonly experienced for 8–12 months after SCI [12]. Through interrupted anterograde signaling, SCI affects the hypothalamus–pituitary–ovary (HPO) axis crucial to normal hormone regulation [12]. Estrogen is produced primarily by developing follicles in the ovary in response to gonadotropic hormones, with the peak of production occurring at ovulation. Decreases in estrogen level result in infrequent menstrual cycles [13]. In addition to controlling reproductive physiology, estrogen also targets the brain [14], significantly affecting individuals' cognitive, psycho-emotional, and depressive status [15]. Estrogen acts through estrogen receptors, a DNA binding transcription factor. Cellular sensitivity to estrogen is determined by the receptor level, and its down-regulation will significantly decrease estrogenic effects [16]. In addition, the changes in steroid concentration is also reflected in the expression level of connexin 43 (CX-43) at tissue level [17]. CX-43 is widely expressed in a variety of cell types including the ovary and involved in the formation of gap junctions.

Gap junctional intercellular connections (GJIC) are necessary for the growth, development, and differentiation of the ovary [18]. GJIC control the coordinated development of somatic granulosa cells, theca cells, and germinal oocytes [19]. GJIC are comprised of connexins, which regulate the spread of intercellular messengers through gap junctions and mediate the paracrine communication of molecules [20]. Crucial to these GJIC, CX-43 is widely expressed in a variety of cells types, including the ovary. In healthy cells, the GJIC are tightly regulated [21]. However, this control is lost under pathological conditions [22–24] and the altered expression of CX-43 in follicles has also been reported to affect ovulation [25].

Estrogen in addition to acting through estrogen receptor, stimulate the endogenous nitrosothiol formation [26]. One of the most abundant endogenous S-nitrosothiols is GSNO, which governs many important physiological functions in vivo and is responsible for nitros(yl)ation of several important proteins [27]. Depletion or reductions in the levels of endogenous nitrosothiols under various neuropathological conditions makes GSNO an important therapeutic agent [28].

Hence, we hypothesized that exogenous administration of GSNO would hasten rats' recovery from SCI-induced transient arrest in estrous cycle and that this positive effect was mediated through the down regulation of CX-43 and up regulation of ERβ, in addition to the inhibition of NFkB.

Materials and Methods

Experimental Animals and Drug

Virgin female Sprague-Dawley rats (225–250 g) were housed individually under a 12-hour light/dark cycle. Food and water were available at all times. The animal procedures for the study were approved by the Institutional Animal Care and Use Committee (IACUC) of the Medical University of South Carolina. Animals exhibiting the same stage of the estrous cycle were subjected to SCI. Following SCI, each animal's estrous stage was determined every day until the animal showed at least two regular 4-day cycles.

Spinal Cord Injury

Contusion-induced SCI was performed under aseptic conditions by following the method described by Bilgen [29]. Animals were anesthetized with a ketamine and xylazine (90 and 10 mg/kg respectively) cocktail injected intraperitoneally. After induction, laminectomy was done at the level of the T8–10 vertebrae. To stabilize the spinal cord during the impact, the T8 and T10 vertebrae were held by stabilizing forceps. SCI had the following parameters: impactor tip—3 mm; duration—85 ms; depth—1 mm. After the injury, the muscles were closed in layers, and the incision was closed with polysorb 4.0. SCI animals were assigned to two groups: one (GSNO) received