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Perturbation of the hypothalamic-pituitary-ovarian axis in experimentally inflicted third degree burns in Wister rats

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ABSTRACT

Burns injury categorized by its severity helps determine the pattern of emergency care management. The pathogenesis and mechanisms through which burn trauma interferes with the female endocrine-equilibrium are still poorly understood. To determine the variations in sex hormone in female rats with third degree burns injury. A total of fifteen adult female regularly cycling Wister rats weighing between 150–200 g were used in the experiments. They were divided into three groups (n=5 rats/ group) designated control, sham, and experimental groups. The control rats were normal usual rats. Third degree burn (TDB) injuries equivalent to 30 % of their total body surface area (TBSA) were inflicted on the skins of the experimental rats. The sham group had 30% of the TBSA shaved with warm water (at room temperature) applied without causing any injury. The rats were sacrificed by cervical dislocation. Blood samples obtained into EDTA specimen bottles. The hormones: Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin (PRL) and Estradiol (E2) were assayed using the Enzyme-linked immunosorbent assay (ELISA) kits. The experimental had a significant increase ($p < 0.05$) in serum E2 and PRL levels compared to sham and control rats. In contrast however, the mean serum FSH and LH levels of experimental were significantly reduced ($p < 0.05$). In the Wister rats inflicted TDB there may have been an alteration in the integrity of hypothalamic-pituitary-ovarian axis that is responsible for the production of these hormones.

Keywords: Burns, Follicle stimulating hormone, Luteinizing hormone, Prolactin, Estradiol.

INTRODUCTION

The release of an ovum by the ovary during ovulation is the most important event in a fertile cycle. The ovulatory mechanism involves the release of two ovarian hormones; Estradiol (E2)

and Progesterone. The former is produced alone by the developing follicle before ovulation while it is produced together with progesterone by the corpus luteum after ovulation. The role of E2 in endometrial cellular proliferation is well established [1]. Estrogen modulates the uterine tissues, provoke endometrial maturation and render endometrium receptive to blastocysts [2]. Progesterone prepares the oestrogen-primed endometrium for implantation of the fertilized ovum.

The cyclic changes in ovarian activity are controlled by the secretion of two gonadotropic hormones released by the pituitary gland, follicle-stimulating hormone (FSH) and luteinizing hormone (LH). Production of these hormones is controlled in turn by an area of the brain called the hypothalamus. Aside these gonadotropins, prolactin (PRL) secreted from special cells of the anterior pituitary gland, the lactotrophs [3] is of significance to the ovarian cycle of the rat. In the early consequence of severe burn injury, an altered reproductive hormonal level reflects a widespread effect on the endocrine system amongst others. Although there are differential responses to burn trauma depending on the gonadal, hypothalamic and pituitary hormone levels at the time of injury and thereafter [4]. Previous studies have shown that burn trauma interfere with the female endocrine balance by involving the hypothalamic centers [5]. Burn traumas if it occurs in the pre-ovulatory phase of the menstrual cycle, disrupt ovulation and hence compromise fertility. The female reproductive cycle is an important variable in the response to trauma hemorrhage [6, 7]. Also studies have shown that the circulating E2 concentration increases 2-fold at 10 days after burn injury in female mice, but no significant increase was observed in male mice [8]. A recent study of severe post burn effects in male rats has shown marked reduction of sperm density and motility, fall in FSH and testosterone which suggests anomalous hypothalamo-pituitary function [9].

Studies have shown that the disruption of ovarian endocrine function has a positive association to the estrous cycle alteration [10, 11]. The concentration of ovarian hormones varies with respect to stages of the estrous cycle. In a succinct review of our erstwhile study, third degree burn (TDB) was observed to cause cessation of estrous cycle at the diestrous phase in rats with supportive histological evidences [12].This present study was extended to assess the parallel peripheral reproductive hormonal findings in Wister rats inflicted with TDB. The knowledge gained would be essential in managing patients that suffered severe burns injury since there are many survivors of burn today due to modern therapeutic intervention.

MATERIALS AND METHODS

Experimental animals

Fifteen adult female Wister rats weighing between 150-200 g having 4-5 days regularly cycling patterns were used for this research. The cycling patterns were confirmed through vaginal smears taken and examined between 8:00-9:00 am every morning for 16 days (4 cycles). They were domiciled in well aerated plastics cages in the Animal House of Anatomy Department of College of Medicine University of Lagos under standardized conditions. Animals were permitted free entrée to pelleted food (Pfizer Nigeria limited) and tap water *ad libitum.* In view of the fact that lighting periodicity plays a principal role in the incidence and duration of the stages of the ovarian cycle [13]. The lighting system was by sunrays (natural light) reflecting through the glass windows of the room providing a photoperiodicity of approximately 12 h light alternating with 12 h of darkness. The rats were allowed to adapt for two weeks before the initiation of the experimental work.

Body weight estimation
The rats were weighed daily before they were sacrificed at the end of experiment. Weighing was done using sensitive weighing machine [14] and values were expressed in grams (g).

Experimental procedure
The animals were randomized into 3 groups *viz:* control, sham and experimental of 5rats/group. Rats in the control group were unshaved (normative regular animals). The other 2 groups had 30% of the total body surface area (TBSA) shaved. Warm water at room temperature was applied to the shaved surface in the rats in sham group without causing any injury while TDB burn injuries were inflicted in the experimental group.

To induce TDB, the rats were anaesthetized with intra-abdominal injection of 7 mg kg -1 body weight ketamine hydrochloride [15]. The back and flank skin of the rats were shaved and later secured into specially designed plastic template whose dimensions gives approximately percentage of body surface areas to be exposed on the right and left lateral flanks. The burn surface areas were decided using Meeh's formula:

$A = 10 \times W^{2/3}$, where: A= area in cm^2, 10 a constant and W=Weight of rats in g [16, 17].

The shaved area were exposed to steam emanating from boiling water of more than 100ºC for a period of 10 seconds [18] to produce a full thickness dermal burn of 30% of the TBSA. This burn technique produces a complete destruction of the nerve tissue, resulting in a burn injury that should not result in pain to the subject (animal). The burns' depths were assessed by physical examination (which entailed checking for pains) and later by histological method [12, 19] on the second and third days. No groups of rats were treated with either antibiotic or anti-inflammatory drugs. They were housed separately in hygienically maintained cages throughout the duration of the experiments.

Sham-"injured" animals were also anesthetized, shaved, held in a plastic template and placed into a room temperature water bath for the same time period. After recovery, animals were returned to their respective cages; retained under barrier conditions in the animal facility.

Autopsy schedule, blood sample collection and storage
In order to eliminate the possibility of irregular cycling caused by pseudo-cyesis, the total duration of study was extended to cover 7 estrous cycles [20, 21]. The rats were sacrificed after 28 days post burn injury. The sacrifice was by cervical dislocation. Blood samples were obtained by left ventricular cardiac puncture immediately into EDTA specimen bottles. The samples centrifuged for 15 minutes at 1000 ×g at 2-8^{o}C within 30 minutes of collection. The plasma removed and assayed at once or stored in aliquot at -20^{o}C in a refrigerator until the time of analysis. The hormones (FSH, LH, PRL and E2) were evaluated using the 96 Enzyme-linked immunosorbent assay (ELISA) test kits for *Rattus norvegicus* following principles described in Uscn Life Science Inc. instruction manual [22].

Test kits for hormonal levels
The test kits E90830Ra 96 ELISA Kit for FSH and E90461Ra 96 ELISA Kit for E2 employ a competitive inhibition enzyme immunoassay technique for *in-vitro* quantitative measurements of FSH and E2 in rat plasma. While the test kits E90441Ra 96 ELISA Kit for LH and E90846Ra 96 ELISA Kit for PRL employ a sandwich enzyme immunoassay for the *in-vitro* quantitative measurements of LH and PRL in rat plasma (Uscn Life Science Inc.) [22].

Statistical Analysis

The results were expressed as Mean (μ) ± Standard Deviation (SD). Test of statistical significance was done by analysis of variance (ANOVA), and unpaired one t tail Student's *t*-test, *p*-value taken as < 0.05.

RESULTS AND DISCUSSION

Table 1: Initial and final body weights before and after burn injury in rats

Group	Initial body weight (g) before burn	Final body weight (g) after burn
Control	200 ± 5.00	210 ± 6.17
Sham	150 ± 4.80	156 ± 5.20
Experimental	180 ± 5.60	178 ± 6.40*

Values are expressed in Mean± S.D; p > 0.05*

Table 2: Variations in Hormone levels in control, sham and experimental rats

Group	FSH (µ/L)	LH (µ/L)	PRL (mµ/L)	E2 (lg/ml)
Control	4.51 ± 1.54	0.29 ± 0.05	4.10 ± 1.43	25.41 ± 2.65
Sham	4.54 ± 1.65	0.24 ± 0.31	8.50 ± 4.82	30.33 ± 10.15
Experimental	3.31 ± 1.31*	0.37 ± 0.03*	16.40 ± 7.04*	63.02 ± 15.71*

*FSH: Follicle stimulating hormone; LH: Luteinizing hormone; PRL: Prolactin; E2: Estradiol Values are expressed in Mean± S.D; * p < 0.05*

In our findings the experimental group with TDB had a significant increase ($p < 0.05$) in serum E2 level (63.02 ± 15.71 lg/ml) compared to sham and the control rats (30.33 ± 10.15 and 25.41 ± 2.65 lg/ml; Table 2). This suggests a disruption in endometrial function causing disparity of estrous cycle [12] and vice-versa. These structural and functional changes of the endometrial lining may lead to failure of implantation and infertility.

The mean serum FSH and LH levels of 3.31 ± 1.31 and 0.37 ± 0.03 µ/L were significantly reduced ($p < 0.05$) after burn injury compared to control (4.51 ± 1.54 and 0.29 ± 0.05 µ/L). The data in the sham group were 4.54± 1.65 and 0.24 ± 0.31 µ/L although showing a non-significant minimal rise in FSH level (compared to control) the general findings show that gonadotropins levels were low in the early stages following burn injury. The gonadotropins responsible for stimulating ovulation are produced in a specific pattern. Normally, the hypothalamus generates pulses of gonadotropin-releasing hormone (GnRH) that stimulates the pituitary to produce gonadotropins. Gonadotropins stimulate the ovaries to produce estrogen [23]. Since TDB interfered with the endocrine equilibrium, it therefore may have caused a block/impairment to the hypothalamic-pituitary-ovarian (HPO) axis via interference in the signaling pathway resulting in a negative feedback on the secretion of gonadotropins [24]. A combination of low gonadotropins and estradiol is indicative of a compromised pituitary/hypothalamic level.

There was a significant rise in PRL levels (hyperprolactinaemia) in the experimental (16.40 ± 7.04 mµ/L; $p < 0.05$) group in contrast to sham and control (8.50 ± 4.82 and 4.10 ± 1.43 mµ/L; Table 2). Some well documented features of hyperprolactineamia include amenorrhea, anovulalory cycles and increased estrogen hence a common endocrine cause of infertility in female [25, 26]. Also the induction of untreated TDB may also imposed physical and 'emotional' stress. Stress can suppress the release of GnRH from the hypothalamus [27]. This represents temporary alterations in the stress centers of the rats brain that control secretion of GnRH.

This ultimately interferes with normal cycles or may have brought about the infertility in the experimental rats. In addition, serum prolactin tend to increase based on the severity of the burn because prolactin is a stress hormone.

The body weight decrease in the experimental rats compared to sham and control (Table1) was non-significant ($p < 0.05$). This may not have played any role in integrity of the female reproductive endocrinology as studies have shown that only a recent substantial weight loss of 10 % of body weight has been proven to disrupt gonadotropins pattern of secretion and affect ovulation [28].

In conclusion the evaluated facts show that TDB injury can possibly be the etiology for infertility amongst female burn survivors by serving as ovulation blocker and inhibit fertility.

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