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FREE RADICAL BOMBING OF SPERMATOZOA IN SPERMATIC GRANULOMA : AN ATTEMPT TO PREVENT AUTOIMMUNE SWITCH-ON

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SUMMARY Even though vasectomy-associated reactions in subjects are established, the biochemical attributes of the granuloma cells enabling them to potently dispose of the accumulating spermatozoa remain unelucidated. This study demonstrates a careful "free-radical bombing" within the granuloma interior to efficiently disintegrate all the structural elements of the sperm cells, especially the proteins. A free radical triggered and instantaneous protein-disposal could be important in avoiding early post-vasectomy autoimmune triggering.

INTRODUCTION Vasectomy is a frequently used means of male contraception and thus the nature of the local and systematic changes induced by it still continue to be of immense interest. Different aspects of postvasectomy syndromes have been studied extensively and reports are available on testicular lesion [1], defective spermiogenesis [2], "gangrene" formation [3], phagocyte-accumulation [4] and appearance of new series of antisperm autoantibodies [5]. One of the delayed consequences of vasectomy is the formation of granuloma, the irregular and spherical structures with flexible wall surrounding a semisolid core [6] which might do some beneficial [7] or harmful [8] acts on male reproductive system. Histological investigations reveal the dominance of immune responsive cells in

Abbreviations: SOD = Superoxide dismutase; $O_2^{\cdot -}$ = superoxide anion radical; HBSS =Hank's Balanced Salt Solution; PBN = N-t-butyl-O phenyl nitrone; DDC = Diethyl dithio carbamic acid; EPR = Electron paramagnetic resonance.

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granuloma suggesting them to be the postvasectomy immune-modulators [9]. Presence of degenerating sperm in granuloma [10] makes it appear that they could also act as a "cell degenerating module" by reducing the load of dead, stagnant and damaged sperm from the granuloma cores and relieving the distended system by releasing excessive hydrostatic pressure [11]. Although these secondary reactions of immune response are well understood, no biochemical explanation has been proposed to explain the potent and efficient sperm disposal attributes of granuloma interior. This study examines the relevance of a superoxide theory of sperm disintegration and disposal in the granuloma core.

MATERIALS AND METHODS

Reagents : Trizma base, Trizma HCl,Triton X-100 and Diethyl dithio carbamic acid were purchased from Sigma Chemical Company Inc. USA., PBN and Mal Net were from Aldrich, Milwaukee, WI. Pyrogallol was of Loba Chemie, India and all other chemicals were of reagent grade.

Animals : Adult male albino rats [Wistar strain] of the age of 3 months housed in temperature [27 ± 1°C] and light [14 h light : 10h dark] controlled rooms were used for this study.

Vasectomy : For bilateral vasectomy, the method described by Flickinger et al [8] was followed. Vasectomized and sham operated animals were grouped into two and caged separately for next three months. After three months vasectomized animals were sacrificed by cervical dislocation and vas granulomas from both ends, i.e., proximal end [epididymal side] and distal end [urinary bladder side] were taken out surgically. Entrapped sperm were washed out carefully. For control, pieces of vas deferens were taken from sham operated rats.

Assay of superoxide dismutase : SOD was extracted from pre weighed tissues according to the method detailed elsewhere [9]. The enzyme activity was assayed after Marklund and Marklund [1974] [10]. All the calculations were made as per milligram of fresh tissue weight.

Spin trapping protocol for superoxide radical: 890 µl of tissue suspension [made in HBSS] was incubated with 100 ul of PBN and 10 µl of 1×10^{-3} M DDC for half an hour at 30°C. After incubation , 25 µl aliquots were transferred to glass capillaries [Top syringe Manufacturing Company, India] and one end was flame sealed taking care not to warm the suspension. EPR spectra of the PBN-superoxide radical adduct were recorded on a varian E-104 spectrometer equipped with TM_{110} cavity [11].

Assay of rotational correlation time from spin label study : Sperm from granuloma and vas deferens was flushed out in HBSS buffer solution [pH 7.2] and washed thrice. Suspensions were incubated at 37°C with Mal Net spin label for 20 minutes following another 10 minutes with $NiCl_2$ [50mM]. After incubation 25 µl aliquot was filled into the glass capillaries to seal one end with heat without warming

the sample. EPR spectrometer settings and scannig were done according to the standard method stated elsewhere [12]. Membrane fluidity was measured as the inverse of the Tc value.

Statistical analysis: Statistical analysis was computed using "STATGRAPHICS" version 2.1 [STSC] Inc. 1985,1986.The degree of variance of the results of each group was computed with that of the preceding group by subjecting them to a one way ANOVA. Each experiment was repeated three times.

RESULTS AND DISCUSSION The nett oxiradical stress in the granulomas developed after an experimental vasectomy was studied by quantifying the superoxide production as well as the total SOD activity. The superoxide production was augmented in the proximal granuloma [$p < 0.0007$] when compared with the control preparations [Figure 1] The granuloma developed on the epididymal side [proximal] generated superoxide at higher levels than that at the distal end [$p < 0.010$]. Interestingly, the total superoxide activity in both these granuloma

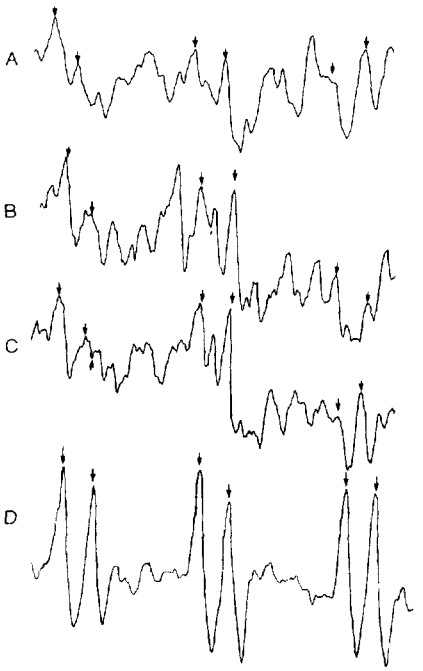

Figure 1. Showing the EPR spectra of PBN-superoxide adducts when $O_2^{\cdot -}$ radical generated from vas deferens [A], Proximal granuloma [B] and Distal granuloma [C]. Reference spectrum of $O_2^{\cdot -}$-PBN adduct [D] was recorded using pyrogallol autoxidation system.

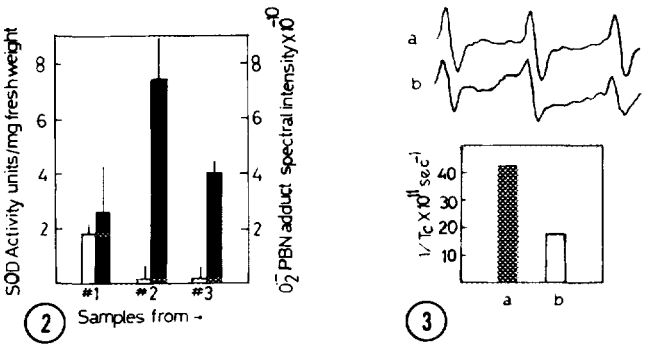

Figure 2. Changes in SOD activity level and Superoxide generation intensity in vasectomized and sham - operated rat after three months of operation. ▓▓ Superoxide generation intensity. ▭ SOD activity. # 1 = vas deferens from sham-operated group. # 2 = Proximal end granuloma and #3 = Distal end granuloma. The values shown are mean ± s.e.m of at least three replicates taken.

Figure 3. Showing the changes in the 1/Tc [a measure of membrane fluidity] values of Mal Net spin label in the membrane of sperm from vas deferens of sham operated [a] and from spermatic granuloma of vasectomized animal [b]. EPR spectra shows the comparative rigidity of granuloma entrapped sperm membrane [b] rather than a sham operated one [a]. ▓▓ Sham operated group. ▭ Vasectomized group. The values shown are mean ± s.e.m of at least three replicates taken.

preparations was considerably lower [$p<0.0009$ for proximal end and $p < 0.010$ for distal end granuloma] as compared to respective control (Figure 2)

Mal Net was used to check the mobility of thiol containing proteins within the membrane of sperm, collected from granuloma core. A drop [$p<0.025$] in the rotational mobility [1/Tc] of Mal Net attached to the membrane proteins of spermatozoa from proximal granuloma signified the elevation of local rigidity and loss of membrane integrity. In the distal granuloma, the sperm count was too low to measure rotational properties of membrane proteins [Figure 3].

The results presented in this paper show that the spermatozoa within the granuloma become prone to the oxidative action of malignant amount of oxygen radicals, facilitated actively by the diminution of a potent quenching factor,i.e.,the SOD activity. The activity of total SOD has been reported to low in several transplantable mouse, rat and hamster tumors [13]. Some chromosomal abnormalities in chromosome number 21 in

human causes some syndromes like Bloom's syndrome, Falconi's syndrome, etc. enhance the risks of malignant diseases due to the low profile of SOD activity [14]. In paraquat-related diseases, superoxide production is attributed to be the main causative agent [14]. "Bacterial killing" mechanism is another oxygen radical-dependent process, where the "Respiratory burst" destroys the microbes within the phagocytic vacuoles. A few types of phagocytic and macrophagic phenomena for the disposal of spermatozoa confined in granuloma and postvasectomy cauda have been reported [4]. Another view postulates the regional lymphatic system as the "burial site" for the postvasetomized spermatozoa [15]. No search had so far been directed to probe into the mystery of free-radical mechanism of spermatozoa-breakdown within the core of granuloma, similar to the situation prevailing in the case of injured tissue-rejection and clearing phenomena which come about with the changes in free radical homeostasis and alteration of SOD activity level [16]. This study has revealed a local"respiratory burst" in the granuloma cells detonating a free radical "bomb" which could shatter down the accumulating sperm cells. We feel that the granuloma cells have acquired their "free radical artillery" to efficiently disintegrate all the structural elements of the sperm cells , especially the proteins, with minimal chances given for the development of an autoimmune response.

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