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A dense matrix of epididymal origin to process and remove defective spermatozoa: Observation in AFB1-treated rat

Faisal Kunnathodi and Mohammad A. Akbarsha

Department of Animal Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli-620 024, India

Abstract

When male Wistar rats were treated with aflatoxin B1, more than 50% of the spermatozoa were defective. Strangely, about 10% of these spermatozoa were embedded in a dense matrix. TEM observation revealed that a fairly high percentage of such spermatozoa had the outer membrane, mitochondrial sheath or fibrous sheath, depending upon the level along the sperm which was cut in the transverse sections, and even the microtubule doublets in various numbers, in that order, were missing. In a few rare cases ODFs alone, in varying numbers, remained embedded in the dense matrix. This is a clear case of special provision for processing and removal of defective spermatozoa produced due to the toxic insult. We have produced evidence to the effect that the source of the matrix in which the spermatozoa get embedded and undergo disintegration is the epididymosomes, secreted in aprocrine fashion by the epididymal epithelium of the initial segment to caput. This reveals yet another aspect of versatility of the epididymis.

Key words: Defective sperm, toxic insult, dense matrix, sperm disintegration.

Introduction

Serious concerns have been expressed in the recent past regarding the deterioration of human and animal male reproductive health (Carlsen et al., 1992). The major causative factors include environmental toxicants and dietary toxins. One of the manifestations here is spermatotoxicity, resulting in oligo-, astheno- and / or teratozoospermia or combinations of two or more of these manifestations. Several abnormal sperm morphologies produced thus are known in the literature, and newer abnormal morphologies such as extrusion of one or more outer dense fibers (ODFs) along with the associated microtublule doublets on exposure to aflatoxin B1 have been reported (Faisal et al., 2008a). Aflatoxins, naturally occurring food contaminants, are toxic metabolites produced by the molds *Aspergillus flavus, Aspergillus parasiticus, etc*. Aflatoxins can cause serious health problems such as carcinogenesis, mutagenesis, growth retardation, immune suppression, etc. Our laboratory has been concerned with male reproductive toxicological evaluation for several years and we found disruption of spermatogenesis (Faridha et al., 2006, 2007), induction of meiotic micronuclei in spermatocytes (Faisal et al., 2008b) and production of defective spermatozoa (Agnes and Akbarsha 2001) in Swiss mice treated with AFB1, the most potent aflatoxin. We found yet another manifestation in AFB1 treated rats where in the spermatozoa in sections were missing ODFs and associated microtubule doublets comparable to those reported by Faisal et al. (2008a) but several of them remained embedded in a dense matrix. We examined the matrix-embedded spermatozoa and found yet another manifestation in abnormal sperm morphology. Here in we describe these manifestations and also suggest a role for the epididymosomes (Hermo and Jacks, 2002; Frenette et al., 2006) in the genesis of such matrix-embedded spermatozoa.

Materials and Methods

The experiment was approved by the Institutional Animal Ethics Committee. Twelve 90 day old Wistar strain male rats (200-225g body weight) were divided into two groups of six each, one the control and the other experimental. The rats in the treatment group were injected with AFB1 (Sigma, St Louis, MO, USA), dissolved in olive oil, at a daily dose of 20 µg/kg body weight through *intra-muscular* route for 55 days, the duration of one spermatogenic cycle, and those in the control group were administered with olive oil alone. At the end of the treatment the animals were dissected under sodium pentobarbital anesthesia and the epididymides were removed. The cauda

Correspondence to be addressed to: Dr. M.A. Akbarsha, Ph.D., Email: akbarbdu@yahoo.com

epididymidal spermatozoa were subjected to counts using Neubaeur counting chamber, assessment of motility using hanging drop preparation, and observation of morphology using Giemsa's stained smears. Tissue slices of cauda epdididymides, fixed in 2.5% glutaraldehyde and post-fixed in 1 % OsO_4, were embedded in thin viscosity resin. Ultrathin sections were stained in uranyl acetate and lead citrate and observed in a Phillips 201C transmission electron microscope (Amsterdam, Holland).

Results

Most of the spermatozoa from the rats in the control group had normal counts, motility and morphology, whereas in AFB1 treated rats the sperm count was decreased and motility was impaired (Data not presented). Further, more than 50% of the spermatozoa showed abnormal morphologies of various kinds. Many of these spermatozoa were embedded in a dense matrix (Fig. 1). We became curious and made a thorough screening of the manifestations of the matrix-embedded spermatozoa when we found that in a fairly high percentage of such spermatozoa the ODFs were intact but the outer membrane in many sperm and also the mitochondrial sheath or fibrous sheath, depending on the level along the sperm which was cut in the transverse sections, and even the microtubule doublets in various numbers were missing. In a few rare cases the ODFs alone, in varying numbers, remained embedded in the dense matrix (Fig. 2).

In a search for the source of the matrix in which the spermatozoa get embedded, we found the epididymal epithelium of the initial segment to caput to produce epididymosomes profusely (Fig. 3). The epididymosomes arrived at the lumen (Fig. 4), formed into conglomerates (Fig. 5) and spermatozoa got entangled in it (Fig. 6).

Discussion

We have reported recently extrusion of ODFs in varying numbers and the respective associated microtubule doublets in sperm of rat treated AFB1 and traced the origin of this sperm abnormality to problem in the mitochondrial sheath caused due to the treatment (Faisal et al., 2008a). In this study we found two or more epididymal spermatozoa embedded in a dense matrix and such spermatozoa were undergoing disintegration to varying degrees starting with the outer membrane and then the mitochondrial sheath/ fibrous sheath, microtubule doublets and ODFs, in that order. Two aspects of these manifestations are intriguing: i) the purpose that would be served by spermatozoa getting embedded in a dense matrix, and ii) the source of the dense matrix. A thorough search of literature revealed one publication earlier by Cooper and Hamilton (1976) on the occurrence of aggregates of spermatozoa in a dense matrix in the cauda epididymides and vas deferens of rat, mouse, guinea pig and golden hamster. These authors have explained the abnormal morphological features of the spermatozoa, as observed in our study, as an aspect of disintegration or dissolution of defective spermatozoa. Adopting this interpretation, we are led to conclude that AFB1 treatment is causative of defective spermatozoa. In search for the source of the dense matrix, we found profuse discharge of epididymosomes from the epithelium of the proximal segments of the epididymis in AFB1-treated rats. Disintegration or dissolution of the matrix-entangled spermatozoa, and not those lying free in the epididymal lumen, suggests that the matrix is a provision of hydrolytic enzymes that would act on the defective spermatozoa that are matrix-embedded, sparing the others that lie free in lumen.

Epididymosomes are apocrine secretions from the epithelium of epididymis (Frenette et al., 2006). Epididymosomes are associated with a complex mixture of proteins (Thimon et al., 2008) and attributed with roles in transfer of proteins to sperm surface towards their post-testicular maturation (Saez et al., 2003). We have produced evidence here for profuse discharge of epididymosomes when the lumen abounds with defective spermatozoa in the context of aflatoxin toxicity, more profuse than in the control rats, and this coincides with the abundance of matrix-entangled sperm in the epididymal lumen. We suggest that the epididymosomes in this context are concerned with contributing the dense matrix and the enzymatic mechanism for degradation/dissolution of the defective spermatozoa, thereby excluding the normal sperm from the enzymatic degradation, which is another aspect of versatility of epididymis.

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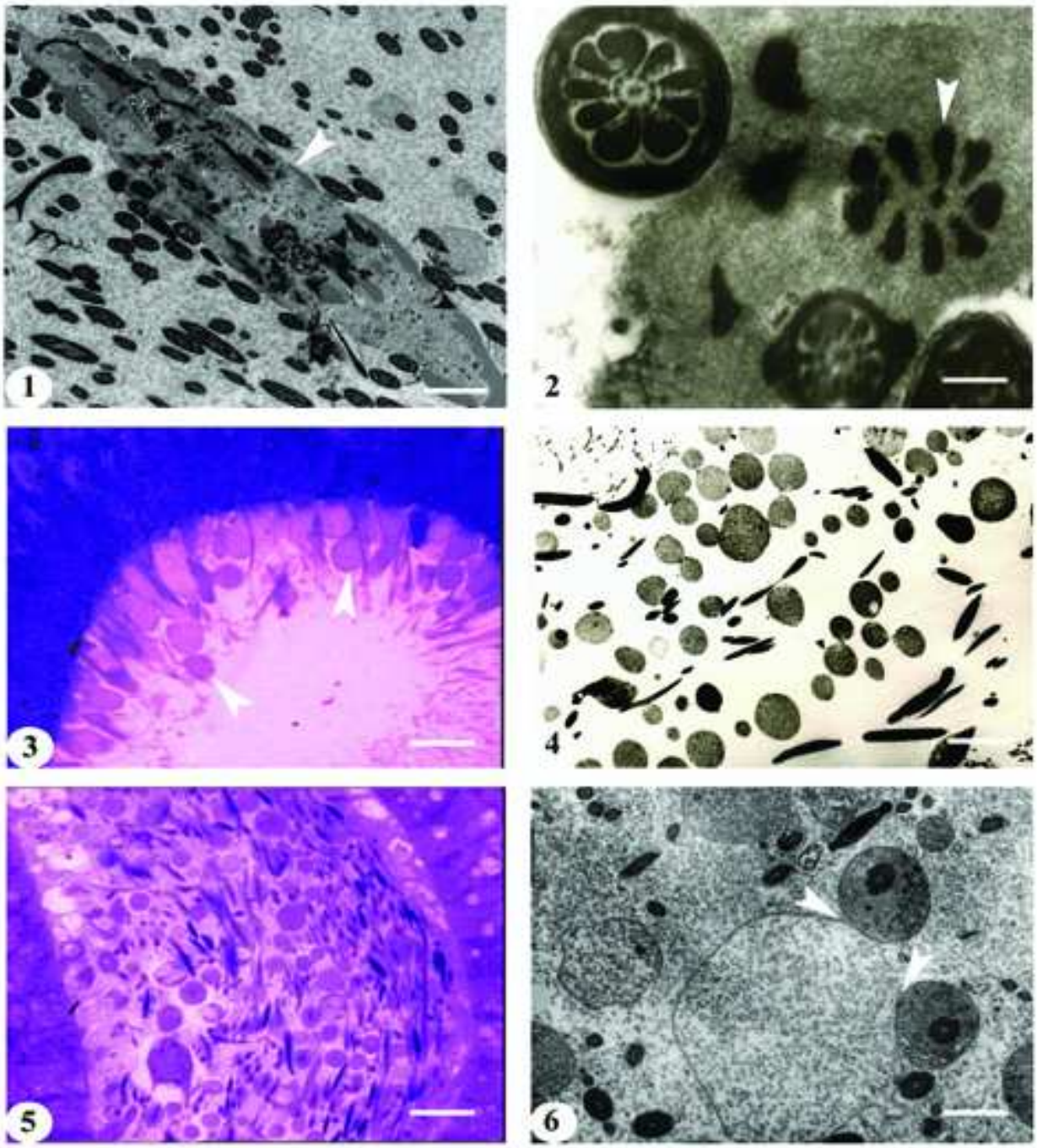

Fig.1. TEM showing matrix-embedded spermatozoa (arrowhead) undergoing disintegration. Scale bar: 5.6 µm.

Fig.2. TEM showing disintegration of matrix-embedded spermatozoa. Arrowhead points to a sperm whose outer membrane is missing. Close by are two ODFs alone of another sperm. Scale bar: 1.2 µm.

Fig.3. TBO-stained semithin section of initial segment epididymidis of AFB1 treated rat showing profuse discharge of epididymosomes (arrowheads). Scale bar: 20 µm.

Fig.4. TEM showing epididymosomes in the epididymal lumen. Scale bar: 5.6 µm.

Fig.5. TBO-stained semithin section of intermediate zone epididymidis of AFB1 treated rat showing fusion of epididymosomes towards formation of the dense matrix. Scale bar: 20 µm.

Fig.6. TEM showing association of epididymosomes with defective spermatozoa (arrowheads). Scale bar: 1.2 µm.

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