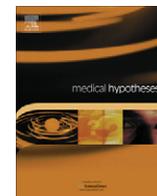


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Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

Adriamycin induced spermatogenesis defect is due to the reduction in epididymal adipose tissue mass: A possible hypothesis

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ARTICLE INFO

Article history:

Received 13 August 2011

Accepted 23 October 2011

Available online xxx

ABSTRACT

Adriamycin is an anthracycline antibiotic used as anticancer drug since past few decades. Though effective against cancer, it is cardiotoxic, nephrotoxic, hepatotoxic and also toxic for reproductive system. Although a number of potential toxic mechanisms have been identified following exposure to adriamycin, the major pathogenic mechanism appears to be the generation of toxic reactive oxygen species (ROS). Animals treated with adriamycin have shown a decrease in total sperm count. This implies that adriamycin impairs the process of spermatogenesis. Epididymal white adipose tissue (EWAT) is necessary for normal spermatogenesis, and decrease in the EWAT causes disturbance in spermatogenesis. Factor X is an unknown molecule synthesized by EWAT that plays crucial role in spermatogenesis. Adriamycin inhibits Kruppel-like factor 4 (KLF-4) and thus downregulates the adipogenesis process needed to maintain the EWAT mass. Apart from adipocytes, KLF-4 and peroxisome proliferator-activated receptor gamma (PPAR- γ) are also found in spermatogonium and testis, implying its vital role in spermatogenesis. Adriamycin treatment inhibits KLF-4 and thus PPAR- γ in EWAT and spermatogonium. Reduction of EWAT might cause a decrease in Factor X level. Declining of Factor X level, KLF-4 and PPAR- γ together will lead to disturbance in spermatogenesis process.

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Introduction

Cancer is a deadliest disease taking many lives every year. Chemotherapy is one of the strategies used in cancer treatment. Adriamycin is an anthracycline antibiotic used as a chemotherapeutic agent to treat many types of cancer including bladder, breast, head and neck cancer and leukemia [1]. Like all other anthracyclines, adriamycin acts by intercalating DNA. Adriamycin is extensively known for its cardiotoxic effect [2], it also causes toxicity in other organs like kidney [3], liver [4] and testis [5–7]. It causes adverse effect in the reproductive system and, a decrease in total sperm count has been reported by many researchers [5–7], which explains that adriamycin is involved in the distress of spermatogenesis process. All the reports published till date explains the generation of ROS as the cause for the toxic effects of adriamycin [8]. However, in this current article we propose a new mechanism that may be involved in adriamycin mediated suppression of spermatogenesis.

Adriamycin and spermatogenesis

Although adriamycin have high antitumor efficacy, but still the use of adriamycin in clinical chemotherapy is limited due to diverse toxicities, including testicular toxicity [5–7]. Adriamycin treatment shows constant dose dependent reductions in testis, epididymis and seminal vesicle weights [9]. Testicular and epididymal sperm count decreased following adriamycin treatment [9]. Sjoblom et al. [10] reported that adriamycin causes an increase in apoptosis at specific stages of seminiferous epithelial cycle and the most sensitive cell types are type A3-4 spermatogonia, preleptotene, zygotene and early pachytene spermatocytes.

Role of KLF-4 and PPAR gamma in spermatogenesis

Kruppel-like factor 4 (KLF-4)

Kruppel-like factor 4 is a transcriptional factor encoded by *klf-4* gene; it has been an indicator of stem cell capacity [11]. KLF-4 is involved in numerous functions like proper differentiation of keratinocytes, epithelial cells of the tongue [12], goblet cells in the colon [13] and the gastric epithelium [14]. KLF-4 has also been

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shown to be an important regulator of the developmental potency of pluripotent cells [11,15,16].

klf-4 mRNA is strongly expressed in post-meiotic germ cells of the mouse testis [17] and also present in the prepubertal mouse testis [18], i.e., before meiotic and post-meiotic germ cells appear, indicating that other cell types besides spermatids also express KLF-4. Behr et al. [17] described that the KLF-4 protein distribution in the normal human testis, in testicular biopsy samples exhibiting round spermatid maturation arrest which suggest a strong role of KLF-4 in spermatogenesis as well as testicular physiology.

PPAR- γ

PPAR- γ belongs to families of nuclear receptor protein that functions as transcriptional factors regulating various genes involved in lipid metabolism. PPAR- γ mainly known as one of the master regulator in adipogenesis [19]. However, it is also developmentally expressed in both differentiating germ and sertoli cells; where it is involved in regulating the pattern of expression of key lipid metabolic genes in sertoli cells [20] which also indicate that, PPAR- γ plays an important role in spermatogenesis.

KLF-4 and PPAR- γ are important for maintaining epididymal fat mass

KLF-4 is a new candidate in the field of fat biology, which came to light very recently. KLF-4 binds directly to the CCAAT-enhancer binding proteins beta (C/EBP β) promoter and transactivates it [21] in response to cAMP. C/EBP β controls a number of genes that are essential for adipogenesis including PPAR- γ . C/EBP β is transiently induced during the early stages adipogenesis. Murine embryonic fibroblasts (MEFs) from mice lacking both C/EBP β and δ show impaired adipocyte differentiation capability in response to adipogenic stimuli, and in addition, the epididymal fat pad weight of

surviving adult C/EBP β ^{-/-}C/EBP δ ^{-/-} mice was significantly reduced compared with wild-type mice [22]. In contrast, ectopic expression of C/EBP β and δ in 3T3-L1 preadipocytes promotes adipogenesis, even in the absence of adipogenic stimuli [23,24]. Hence it may be realized that KLF-4 activates C/EBP β which then promotes adipogenesis, by inducing the expression of the 'master' adipogenic transcription factors like C/EBP α and PPAR- γ . Therefore, KLF-4 and PPAR- γ are important for maintaining the epididymal fat mass (Fig. 1).

EWAT and spermatogenesis

EWAT produces a locally acting factor responsible for maintaining spermatogenesis. A very recent study showed that removal of the epididymal fat pad interrupts spermatogenesis and increases double the amount of Follicle Stimulating Hormone (FSH) concentration but does not affect testosterone, Luteinizing Hormone (LH) concentration, or mating behavior [25]. Transplantation of the excised epididymal fat pad to a subcutaneous site did not restore spermatogenesis. Removal of comparable amounts of white adipose tissue from other sites (inguinal) showed no effect, disproving the idea that the effect is due to a decreased energy supply and/or the need for some minimal amount of fat. The authors concluded that it might be due to the presence of a local, but currently unidentified, growth and/or nutritive factor from EWAT that promotes spermatogenesis.

The relationship between adriamycin and KLF-4

In a study to explain that p53 response to DNA damage is regulated by KLF-4, the authors have shown that cells treated with adriamycin inhibited the expression of KLF-4 in a time dependent manner [26]. This study clearly explained that adriamycin directly downregulates the KLF-4 expression.

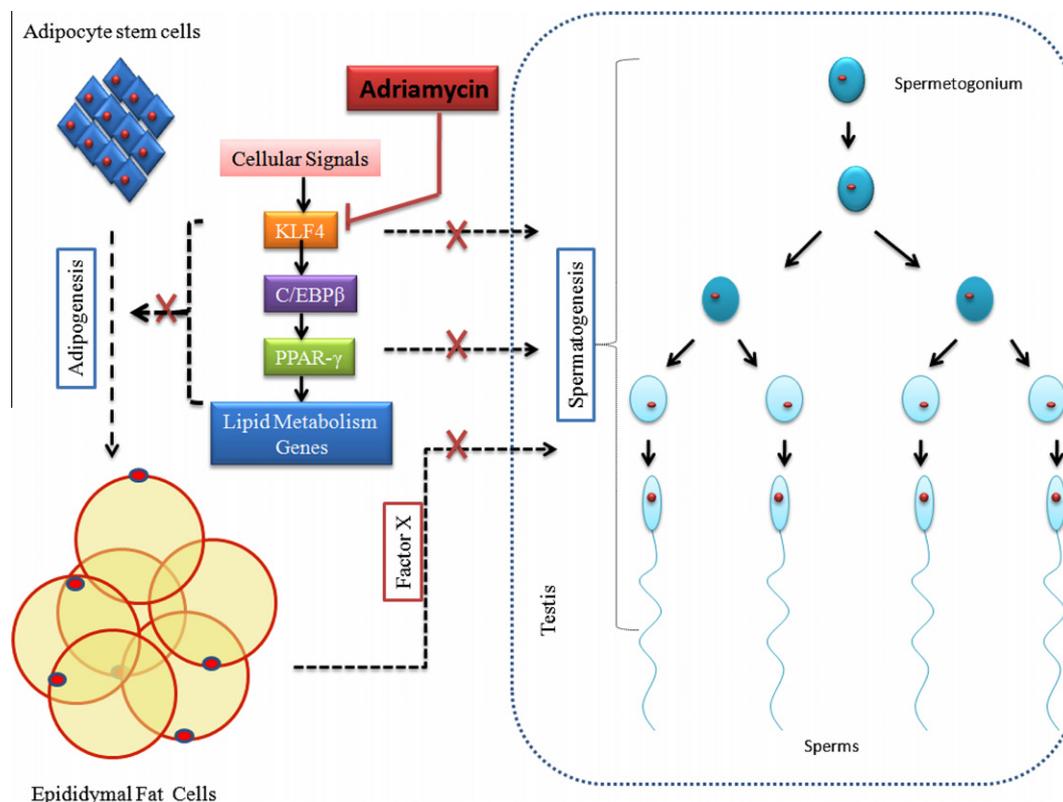


Fig. 1. KLF-4 which is involved in spermatogenesis and adipogenesis get inhibited due to adriamycin treatment. The cross marks in the figure shows the possible mechanisms that get disturbed after inhibition of KLF-4 by adriamycin. Factor X is an unknown molecule that is secreted by epididymal adipocyte and is important for spermatogenesis.

Hypothesis

Epididymal fat pad is essential for spermatogenesis and reduction of the fat pad influences spermatogenesis in a negative manner. KLF-4 regulates the expression of CEBP beta, which governs the expression of adipogenic genes. Inhibition of KLF-4 downregulates the expression of essential adipogenic master regulators including PPAR- γ . Adriamycin is able to inhibit KLF-4, therefore, we hypothesize that adriamycin downregulates the expression of KLF-4, due to which there may be a disturbance in fat cell physiology specially EWAT. Inhibition of KLF-4 may lead to decrease in the epididymal fat pad mass which may be the reason for reduction and upset spermatogenesis in animals treated with adriamycin. This effect might be the cause of impairment of reproductive ability of adriamycin treatment in males. Additionally it can be expected that treatment of animals undergoing adriamycin therapy with PPAR- γ agonist like thioflitazones may be helpful in restoring the epididymal fat pad mass and thus may help in improving spermatogenesis as a therapeutic strategy.

Conflict of interest statement

No conflict of interest.

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