Immunomodulatory Treatment for Infertile Men with Antisperm Antibodies

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Fertility and Sterility, 2004; 81(3):S7-31: pp. 20.

The results were presented on the

52nd annual meeting and postgraduate course of the Pacific Coast Reproductive Society.

April 28-May 2, 2004. Rancho Mirage • California, USA. ID:/Article No: 943

ABSTRACT

One of the important categories of subfertility or infertility in humans may be immunological diseases mediated by antisperm antibodies (ASA). Blood sera from 35 infertile men of different age with ASA positive ELISA test were examined for the serum level of ASA before and after treatments with 1α,25-dihydroxy-Vitamin-D3 and Dexamethasone. We treated 18 infertile men with Vitamin-D3/Dexamethasone during 30 days, 9 infertile men with Dexamethasone only during 30 days and 8 infertile men received no treatment. All the patients showed poor parameters of spermogram and high level of ASA serum concentration (>75 U/ml). Serum concentration of ASA in non-treated group (313 U/ml), Dexamethasone only treated group (288) and Vitamin-D3/Dexamethasone treated group (124 U/ml) were significantly different. Serum level of ASA in Vitamin-D3/Dexamethasone treated group was significantly lower compared to the level before the treatment ($P<0.01$). These findings could be explained by immunosuppressive and immunomodulatory effects of Dexamethasone and Vitamin D3 treatment.

Key Words: antisperm antibody, dexamethasone, infertility, vitamin D3.

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INTRODUCTION

Autoimmunity to sperm may occur because sperm cell antigens are first expressed during sexual maturation, long after the prenatal period when immunological self-tolerance is induced [1,2]. Protection against autoimmunity is provided by the blood-testis barrier composed predominantly of Sertoli cells isolating the tubular content from the vasculature and limited lymphatic drainage of the testis [3]. Several other immunoregulatory mechanisms also play a significant role in the prevention of anti-sperm immunity, such as immunosuppressive factors of seminal plasma, as well as both systemic nonspecific and specific factors (immunoregulatory cells, cytokines, absence of co-stimulatory molecules expression etc.) [4]. In some cases, auto-immunization with sequestered sperm molecules happened after disruption of blood-testis barrier by disease and/or injuries [5,6]. Generally, humoral immune response such as ASA formation can be induced primarily during infectious and noninfectious inflammations, or by obstruction of testicular efferent duct [1,7]. The ASA was also induced after accidental and/or surgical injury of testicles, exposure to very low temperature or cryptorchism [5,6]. Subsequently, infertility can result from antibodies directly binding the sperm, or from aspermatogenesis due to allergic orchitis. A similar phenomenon occurs in vasectomized laboratory rodents and man [5]. Most affected individuals develop epididymal sperm granulomas and testicular degeneration associated with the formation of antisperm antibodies [5,8]. Finally, the presence of ASA reacting with antigens on the sperm considered typical and specific immunological infertility [8].

ASA can impair fertilization by several mechanisms. They can interfere with sperm motility by immobilizing or agglutinating the sperm, or interfere with sperm-cervical mucus interaction and disturb sperm transport [9]. ASA mediated impairment of fertilization can occur as interference of the penetration into the oocyte, and perhaps affect zygote development by impairing early cleavage, or even damaging the implantation process [10]. Whether ASA are involved in pregnancy loss is still debatable as no conclusive evidence is available, so that this subject needs further research [9,10].

Corticosteroids and Vitamin D3 can have profound effects on immune response in mammals on different levels and IgG responses to different antigens [11,12]. Therefore, we speculate that Vitamin-D3 and Dexamethasone can be useful in treatment of infertile, ASA positive patients.

MATERIALS AND METHODS

Thirty-five infertile men with serum level of ASA higher than 75 U/ml (as recommended by the ASA Kit manufacturer) and poor parameters of spermogram comprised three study groups.

Group 1

Eighteen men were treated with Vitamin D3/Dexamethasone combination. Vitamin D3 was administered orally (0.025 mcg/kg of body weight) during 30 days. Dexamethasone was administered during 30 days in dose-decreasing manner. On day one of the treatment, Dexamethasone was administered intramuscularly (im.) in one-day dose 110/mcg/kg. This was followed by one-day dose of 55 mcg/kg administered im. (days 2 and 3). The treatment with Dexamethasone was followed by decreasing oral dose starting with 42 mcg/kg (on day 4 of the treatment) to 7 mcg/kg (on day 30 of the treatment).

Group 2

Nine men treated only with Dexamethasone that administrated during 30 days as well as in group 1.

Group 3

Eight men were not treated and they will be included in treatment in one of further investigations.

Spermogram

The semen analyses were performed according to the guidelines of the WHO (1992) [13]. Sperm count in all groups of patients was analyzed before the treatment and 15 days after treatment. Abstinence time before sperm sampling was 5 days.

Serum Antisperm Antibody ELISA Test

Serum concentration of antisperm antibodies was performed on HUMAN ELISA READER instrument with *Immuno-Biological Laboratories* (IBL) Sperm Antibody Enzyme Immunoassay Kit. The test is based on a non-competitive ELISA. The strips were incubated with diluted sera (1:50) from patients, and after washing steps, were incubated again with peroxidase conjugated anti-human-Ig (IgA, IgG and IgM). Following the final wash and enzyme substrate addition, the developed color was determined using the ELISA reader. Positive results are indicated by ASA concentrations > 75 U/ml in diluted sample of serum as recommended by IBL.

Statistical Analysis

All parameters of 3 study groups were analyzed. P value of less than 0.05 was considered to indicate statistical significance. Calculations were performed with MS Excel ® 2002 by t-test.

RESULTS

Average age of all patients was 35±7. As a possible etiologic factor of ASA presence, we found cryptorchism in 11% (unilateral and bilateral), orchitis in 11%, varicocoela in 26%, accidental trauma in 3%, surgical intervention in 3%, epididymitis in 11% and unknown etiology in 35% of patients. Basic parameters of spermogram and serum level of ASA studied at the time of starting therapy and after 45 days are summarized in table 1.

Table 1 demonstrates basic parameters of spermograms before and after treatment in all 3 groups of infertile men. No significant differences were found recorded in sperm count volume, sperm concentration and percent of cells with normal morphology before and after treatment in 3 studied groups ($P>0.05$). Likewise, no significant differences in motility and viability of spermatozoa were found before and after treatment in group 2 and group 3 ($P>0.05$). Percent of motile ($P=0.021$) and vital ($P<0.01$) spermatozoa in group 1 is significantly higher after treatment in relation to the percent before treatment.

In group 3, no significant changes were found in serum level of ASA in relation to the initial sample and after 45 days ($P=0.54$). Also, serum level of ASA in group 2 did not show significant changes before

and after the treatment (P=0.21). Nevertheless, the level of ASA in group 1 was found to be significantly lower after Vitamin D3/Dexamethasone treatment as compared to the level before the treatment (P<0.01).

Table 1. Basic parameters of spermograms and serum level of ASA before and after treatments (45 days).

		Group 1 Vitamin D3 and Dexamethasone n=18	Group 2 Dexamethasone only n=9	Group 3 no treatment n=8
Volume (ml)	before	2.5±0.7	2.6±0.7	2.8±1
	after	2.6±0.7	2.7±0.5	2.9±0.4
Sperm concentration (10^6/ml)	before	17±9	16±11	17±9
	after	19±9	17±9	19±7
Motile (%) (after 60 min.)	before	34±17	34±5	34±13
	after	45±15	35±7	34±8
Vital (%) (after 60 min.)	before	41±8	37±9	38±6
	after	52±10	39±9	36±8
Normal morphology (%)	before	61±15	64±11	63±6
	after	62±12	62±13	61±8
Serum level of ASA (U/ml)	before	311±77	322±90	317±80
	after	124±40	288±83	313±91

Most frequent side effects of the treatments were gastro-intestinal disorders, increased body weight and slight edemas. In most cases, the side effects were not treated, but in some patients gastro-intestinal disorders like nausea and gastritis were successfully treated with *ranitidine* 150 mg twice daily orally, whereas edemas where treated with *furosemide* 10 mg every 2-3 days orally.

DISCUSSION

Every breakdown of blood-testis barrier and protective immunomodulatory mechanisms may lead to infertility with the autoimmune etiology. In most cases, the autoimmunity on sperm molecules resulting from trauma or infectious disease can generate ASA [1,5,6]. Mechanisms that can provide the autoimmunity and ASA production are micro-environmental acceleration of T-helper-type-1 (Th1) of immunity, enhanced secretion of pro-inflammatory cytokines like IL-1, IFdN-γ, TNF-α, reduced secretion of anti-inflammatory cytokines like IL-10 and TGF-β, up-regulation of MHC and co-stimulatory molecules expression and down-regulation of immune cells apoptotic mechanism [1,4,5,6].

Poor parameters of spermogram, elevated level of ASA and infertility in men are linked with history of cryptorchism, orchitis, varicocoela, epidydimitis and accidental or surgical trauma of male genital tract. Only a few patients have no clear etiologic factor for ASA and infertility, although ASA may form as a result of exposure of sperm antigens to the rectal mucosa, and they have been detected in the sera of a high percentage of homosexual men [14].

The immunosuppressive effects of dexamethasone are multiplex. For instance, the drug suppresses antigen presenting cells, dendritic cells, down-regulates co-stimulatory and MHC molecules expression, as well as T-helper-type-1 cells (Th1) and production of pro-inflammatory cytokines. Dexamethasone has strong suppressive effects on macrophages and T cells, so that the effects can indirectly inhibit antibody production by B cells and proliferation of B cells clones [15,16]. Nevertheless, in our study the drug has no significant effect on ASA level in patients treated with dexamethasone only. Curtis et al. [16] reported similar findings that dexamethasone has no effects on serum level of sperm agglutinating antibody in vasectomized men [16]. Methylprednisolone could increase incidence of pregnancy and live birth rates in infertile couples, albeit the ASA level was not significantly different before and after the therapy [17].

Vitamin D3 inhibits production of monocytes-derived cytokines such as IL-1α, IL-6, and TNF-α. The proliferation of T cells and their release of cytokines such as IL-2 and IFN-γ are also suppressed by Vitamin D3. This occurs partly because of the pre-transcriptional reduction of T cell-activating cytokines production, but also because of a direct effect on the T cells [18]. Although Vitamin D3 has no apparent effect on B lymphocytes, the T cell suppression may indirectly inhibits antibody production by B cells [19]. Vitamin D3 directly inhibits IFN-γ secretion by Th1 clones while it has little effect on IL-4 secretion by Th2

clones. These facts are important due to IFN-γ and IL-2 induce B cells to produce IgG2a while IL-4 and IL-10 induce the production of IgG1 and IgE by B cells [18,19]. These actions of the vitamin D3 suggest that it may have potential therapeutic quality in Th1-mediated autoimmune disease [20]. In addition, VD3 inhibits the ability of antigen presenting cells to induce T cell activation and might involve down regulation of co-stimulatory molecules. The inhibitory effect of VD3 on dendritic cell maturation was comparable to that induced by IL-10, a cytokine which inhibits antigen presenting cells at different levels, including inhibition of IL-12 secretion and MHC molecules expression [18,21].

Synergistic immunomodulatory effects of Vitamin D3/Dexamethasone treatment might be acceptable explanation for significant differences in serum level of ASA in group 1 before and after the treatment. In addition, decreased level of ASA in group 1 probably contributes to a significantly higher percent of vital and motile sperm after the treatment.

There are several techniques of processing semen to select the antisperm antibody-free sperm, or to free up sperm already coated with antisperm antibodies. Collection of the sperm samples directly into a culture medium, followed by rapid washing of the sperm seem to increase the proportion of antibody-free sperma and to improve the fertilization rate for in vitro fertilization and intrauterine insemination. Current techniques are partly efficient, so we suppose that Vitamin D3/Dexamethasone treatment can be used to treat infertile men in IUI, IVF and ICSI.

In conclusion, infertile men with elevated level of ASA and poor basic parameters of spermogram can be treated with Vitamin D3/Dexamethasone protocol with great chance for decreasing the level of ASA. Consequently, significantly increased motility and viability of the spermatozoa as a result of low ASA activity were reported after Vitamin D3/Dexamethasone treatment. ASA may play a mayor role in pathogenesis of male infertility, so that Vitamin D3/Dexamethasone combination may be used as a treatment in different procedures of assisted reproduction. Further study is needed to identify the mechanisms of Vitamin D3/Dexamethasone treatment action(s) in down regulating the ASA level, and to point to real benefits of the treatment.

REFERENCES

1. Naz RK, Menge RC (1994): Antisperm antibodies: origin, regulation and sperm reactivity in human infertility. Fertil Steril 61:1001-1013.
2. Koide SS, Wang L, Kamada M (2000): Antisperm antibodies associated with infertility: properties and encoding genes of target antigens. Proc Soc Exp Biol Med 224:123-132.
3. Setchell BP, Voglamyer JK, Waites GM (1969): A blood-testis barrier restricting passage from blood rete testis fluid but not into lymph. J Physiol 200:73-85.
4. Sainio-Pollanen S, Saari T, Simell O, Pollanen P (1996): CD28-CD80/CD86 interactions in testicular immunoregulation. J Reprod Immunol 31:145-163.
5. McDonald SW (2000): Cellular responses to vasectomy. Int Rev Cytol 199:295-339.
6. Sakamoto Y, Matsumoto T, Mizunoe Y, Haraoka M, Sakumoto M, Kumazawa J (1995): Testicular injury induces cell-mediated autoimmune response to testis. J Urol 153:1316-1320.
7. Almagor M, Dan-Goor M, Hovav Y, Kafka I (1998): Antisperm antibodies in men with psychogenic anejaculation. Arch Androl 41:1-4.
8. Shibahara H, Tsunoda T, Taneichi A, Hirano Y, Ohno A, Takamizawa S, Yamaguchi C, Tsunoda H, Sato I (2002): Diversity of antisperm antibodies bound to sperm surface in male immunological infertility. Am J Reprod Immunol 47:146-150.
9. Marshburn PB, Kutteh WH (1994): The role of antisperm antibodies in infertility. Fertil Steril 61:799-811.
10. Lombardo F, Gandini L, Dondero F, Lenzi A (2001): Immunology and immunopathology of the male genital tract – antisperm immunity in natural and assisted reproduction. Hum Reprod Update 7:450-456.
11. Slack J, Risdahl JM, Valberg SJ, Murphy MJ, Schram BR, Lunn DP (2000): Effects of dexamethasone on development of immunoglobulin G subclass responses following vacci-nation of horses. Am J Vet Res 61:1530-1533.
12.Lemire JM, Archer DC, Beck L, Spiegelberg HL (1995): Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. J Nutr 125:1704-1708.
13. WHO manual, 3rd edition, 1992.
14. Wolff H, Schill WB (1985): Antisperm antibodies in infertile and homosexual men: relationship to serologic and clinical findings. Fertil Steril 44:673-677.
15. Snyder NK, Kramer CM, Dooley RK, Holsapple MP (1993): Characterization of protein phosphorylation by 2,3,7,8-tetrachlorodibenzo-p-dioxin in murine lymphocytes: indirect evidence for a role in the suppression of humoral immunity. Drug Chem Toxicol 16:135-163.
16. Curtis GL, Kahl VA, Ryan WL (1987): Serum sperm agglutinating antibody formation in vasectomized men treated with dexamethasone. Andrologia 19:597-601.
17. Shulman JF, Shulman S (1982): Methylprednisolone treatment of immunologic infertility in male. Fertil Steril 38:591-599.
18. Muller K, Bendtzen K (1996): 1,25-Dihydroxyvitamin D3 as a natural regulator of human immune functions. J Investig Dermatol Symp Proc 1:68-71.
19. Muller K, Heilmann C, Poulsen LK, Barington T, Bendtzen K (1991): The role of monocytes and T cells in 1α,25-dihydroxyvitamin D3 mediated inhibition of B cell function in vitro. Immunopharmacology 21:121-128

20. Bubanovic I (2004): 1α, 25-dihydroxyvitamin D3 As New Immunotherapy In Treatment of Recurrent Spontaneous Abortion. Med Hypotheses 63:250-253.
21. Pichler J., Gerstmayr M., Szepfalusi Z., Urbanek R., Peterlik M., Willheim M (2002): 1α,25(OH)2D3 inhibits not only Th1 but also Th2 differentiation in human cord blood T cells. Pediatr Res 52:12-18.