The Effect of Endogenous Dehydroepiandrosterone Sulphate on Antibody Response to Hepatitis B Vaccine in Neonates

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Abstract

Background: DHEAS, the most abundant steroid secreted by the adrenal cortex, is suggested to have an important role in the development of immune reaction by activating T cell function and increasing antibody response, and has been tried as a vaccine adjuvant in elderly people.

Objectives: We examined the correlation between endogenous DHEAS and antibody response in the neonatal period by comparing the serum DHEAS levels with the amount of antibody response against hepatitis B vaccination in neonates.

Methods: Vaccine was administered to 12 healthy infants within 24 hours of birth (day 0), and blood specimens were obtained on days 0 and 30 for determination of anti-hepatitis B surface antibody concentration and DHEAS levels.

Results: DHEAS levels varied widely (range 0.38–3.70 μg/ml, mean±1SD 2.14±0.98). While we could identify two groups of patients — those with high DHEAS levels (2.90±0.56) and those with lower levels (1.30±0.56) — there was no correlation between DHEAS levels and the antibody response to hepatitis B vaccine (r=–0.05).

Conclusions: In neonates, antibody response to hepatitis B vaccine does not correlate with DHEAS serum levels. These results do not support the usage of DHEAS as a vaccine adjuvant in neonates.

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DHEAS is the most abundant steroid secreted by the adrenal cortex with a half-life of 8–11 hours [1,2]. Serum DHEAS level is a reliable marker of adrenal androgen secretion in both infants and adults [3,4]. During early infancy it is secreted by the fetal zone of the adrenal cortex, which constitutes 80–90% of the adrenal gland. Since the fetal zone undergoes involution in the first year of life, the capacity for DHEAS synthesis and secretion declines during this period. It was recently shown that during acute respiratory illness (acute bronchiolitis), the steroidogenic capacity of the fetal zone is markedly increased in infants younger than 3 months, with significant elevation of DHEAS levels during illness [3].

Accumulating evidence from recent years suggests that dehydroepiandrosterone and its sulphated form, DHEAS, play an important role in the development of immune responses by activating T cell function [5–8]. Their administration as an adjuvant, either systemically or topically, enhanced antibody responses against recombinant hepatitis B surface antigen in mice [7] and against tetanus toxoid in elderly humans [8].

The purpose of the present study was to compare the antibody levels generated following hepatitis B vaccination and serum DHEAS levels in neonates, with the knowledge that at this age group DHEAS levels are higher than later in childhood and that these levels vary considerably among healthy neonates. We hypothesized that patients with higher DHEAS levels would respond with higher antibody concentration following vaccination, reflecting the immunomodulating properties of DHEAS.

Methods

Serum DHEAS levels and anti-HBsAg were determined in 12 neonates. The sera were obtained from patients enrolled in a larger study that examined the safety and immunogenicity of a novel hepatitis B vaccine [9].

Our study group included only healthy infants with a birth weight ≥2,500 g and a 5 minute Apgar score of at least 7. Exclusion criteria included parental alcoholism, drug abuse or concurrent use of medication that might affect immunoresponsivity, and neonates with evidence of disease or receiving medication.

The vaccines were administered to infants within 24 hours after birth (day 0), and blood specimens were obtained on day 0 and day 30 (prior to the next scheduled vaccination).

Vaccine

Bio-Hep-B consists of HBsAg particles of the adw2 serotype, biosynthesized via recombinant DNA technology in engineered Chinese hamster ovary cells harboring the entire hepatitis B surface gene and manufactured according to Good Manufacturing Practice Guidelines of the Food and Drug Administration (USA) by BioTechnology General (Israel). The recombinant HBsAg is secreted into the culture medium as particles harboring the three co-terminal

DHEAS = dehydoepiandrosterone sulphate

HbsAg = hepatitis B surface antigen

surface proteins of HBV, S, Pre S2 and Pre S1, in glycolysated and non-glycolysated form and in proportions that mimic the composition of authentic plasma-derived particles [9,10].

We assessed immunogenicity by determining anti-HBs concentration in serum samples and determined the titers using a commercial radioimmunoassay kit (AUSAB; Abbott Laboratories, Chicago, IL, USA), and calculated by the Hollinger formula in international units per liter (mIU/ml) against a WHO reference standard use.

Serum DHEAS levels were determined by use of a commercial RIA kit (Diagnostic Products Co., Los Angeles, CA, USA). DHEAS levels were determined in two distinct groups of patients: those with either remarkably high or with very low immunological response to hepatitis B vaccine (6 high responders and 6 low responders). Only sera from patients with baseline negative anti-HBs antibody and other negative HBV markers (i.e., anti-hepatitis core antibody and HBsAg) were selected for this study. Both antibody titer and DHEAS levels were determined in the two blood samples obtained from each patient (on day 0 and 30 following vaccination). This study was approved by the Ethics Committee of the Ministry of Health, Jerusalem.

Statistics

Statistical analysis was performed with Student's *t*-test and with Pearson's correlation test.

Results

DHEAS levels and hepatitis B antibody titers were determined in 12 patients.

Immunogenicity

Geometric mean concentration on day 30 ranged from 0.7 to 228.2 mIU/ml. Six patients were high responders to the vaccine (GMC >70 mIU/ml) and the other six were low responders (GMC ≤2 mIU/ml). The difference in GMC between the high responder group and the low responder group was statistically significant ($P=0.006$).

DHEAS levels ranged from 0.38 to 3.70 μg/ml (mean±1SD=2.14±0.98). Six neonates had high DHEAS levels (>2 μg/ml, 2.90±0.56) and the other six had levels ≤2 μg/ml (1.30±0.56). The difference in DHEAS concentration between the two groups was statistically significant ($P=0.0006$). Neonates with high DHEAS levels also showed high levels when their sera were reexamined one month later (correlation between DHEAS levels on days 0 and 30, $r=0.98$, $P<0.0001$, Figure 1).

Comparisons between DHEAS levels and antibody response failed to show any correlation between these two parameters ($r=-0.05$, Figure 2); two of the six high responder neonates and four of the low responders had high DHEAS levels.

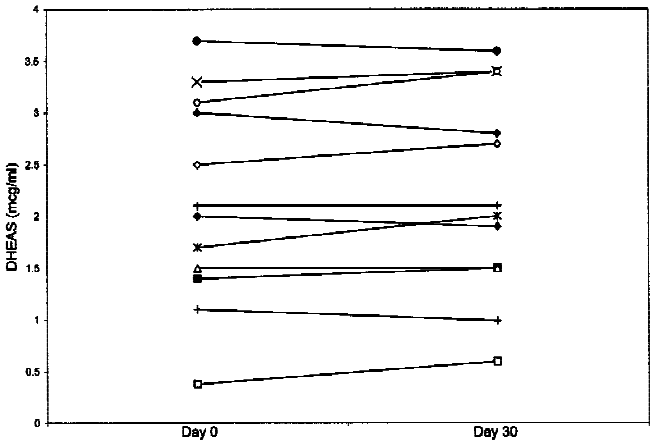

Figure 1. DHEAS levels measured on days 0 and 30 in 12 neonates.

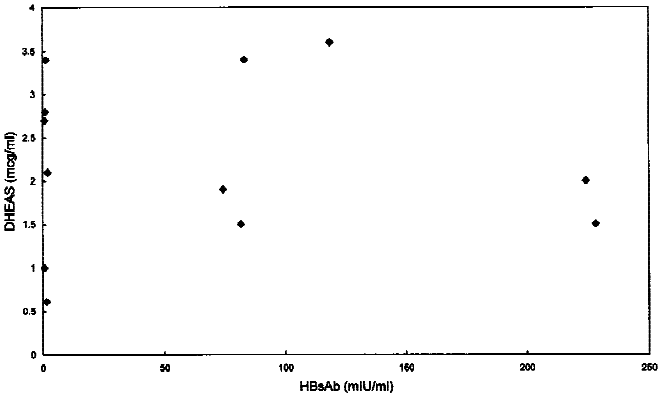

Figure 2. Anti-HBs antibody concentration detected on day 30 in relation to DHEAS levels measured on day 0 in the 12 neonates studied.

Discussion

The effects of DHEAS on the immune system have been documented in both humans and animals; in mice, administration of DHEAS improved the immune response to vaccines such as hepatitis B and influenza vaccine [7].

Addition of DHEAS to *in vitro* culture of human lymphocytes inhibited Epstein-Barr virus-induced morphologic transformation [11], and human immunodeficiency virus replication [12]. Wisniewski et al. [13] reported a positive relationship between the immune status of patients with HIV-related illness and DHEA, leading to the hypothesis that DHEA deficiency may worsen the immune status of HIV-infected patients.

Elderly patients treated with DHEA exhibited an increase in monocytes and natural killer cells, and in both B and T cell mytogenic response, and enhanced release of interleukin-2 and IL-6 in response to *in vitro* mitogen stimulation [5]. A

RIA = radioimmunoassay
GMC = geometric mean concentration
HIV = human immunodeficiency virus
IL-6 = interleukin 6

study in aging adults showed that a one-time supplemental dose of DHEAS with influenza vaccination appeared to enhance the specific human immunodeficiency antibody response to the 1993–1994 H3N2 antigen [14].

The mechanism by which DHEAS exerts its immunological effects is not clear. Consideration has been given to the potential role of an increase in bioavailable insulin-like growth factor-1, which by virtue of its mitogenic effect on immune cell function may mediate the DHEAS effect [5].

These data, coupled with the known variability of DHEAS levels in neonates, led us to design the present study in which we examined the correlation between natural DHEAS level of neonates at the time of hepatitis B vaccination and the antibody titer produced in response to the vaccine. While our study showed a considerable variability in DHEAS levels in the patients studied (DHEAS levels ranged from 0.38 to 3.70 μg/ml), there was no correlation between these levels and the amount of antibody response to the vaccine.

Why did we fail to demonstrate any effect of DHEAS on antibody response to hepatitis B vaccine? It could be argued that DHEAS blood levels at the time of vaccine administration reflect a temporary and transient state. However, the tendency for high or low serum levels of DHEAS prevailed also when DHEAS levels were tested one month after vaccination (correlation 0.98 between the results of the two samples, $P>0.0001$). A possible explanation for the lack of association between natural DHEAS level and specific antibody production is that higher serum levels of the hormone are needed in order to manipulate this arm of the immune system. However, in aging adults, the maximum single dose of DHEAS that could be practically injected subcutaneously (approximately 7.5 mg) evoked a mean DHEAS serum level of 2.3 μg/ml [14], which is lower than the mean neonate level among the high DHEAS levels in our study. Thus it seems that, at least in aged patients, it would be difficult to achieve higher DHEAS levels than those detected in our group.

It is possible that the neonatal immune system is less sensitive to the effects of DHEAS, and that higher levels of this hormone are required in order to manipulate this system. Alternatively, it could be speculated that the relatively high basic levels of DHEAS in the fetus and the neonate already exerted the maximal immunomodulatory effect and, thus, higher levels would fail to achieve any significant additional effect. On the other hand, in the aged, where DHEAS levels are low and sometimes even undetectable, the effect of exogenous DHEAS may be much more prominent.

In conclusion, our results did not show any correlation between endogenous DHEAS levels and antibody response to hepatitis B vaccine in healthy neonates. These results do not support the use of DHEAS as a vaccine adjuvant in neonates. Further studies with a larger number of patients are needed to determine the exact role of DHEAS as an adjuvant in neonatal vaccinations.

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James Dean