Effect of interferon α on high serum androgen concentrations in HIV positive men with Kaposi's sarcoma

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Abstract

Aim—To measure serum androgen concentrations in men with HIV related Kaposi's sarcoma (KS) who had been treated with recombinant interferon (IFN) α-2a to determine the role of androgens on the development of KS lesions.

Methods—32 men with HIV related KS who had been treated with IFN were studied: 24 men in complete KS remission and eight not in remission. Serum androgen concentrations were determined before, during, and after IFN treatment and correlated with clinical remission.

Results—All patients in complete KS remission had lower serum androgen concentrations following IFN treatment: −51% for dehydroepiandrosterone (DHEA) (p < 0.0001); −38% for DHEA sulphate (p < 0.002); −39% for androstenedione (p < 0.002); and −44% for testosterone (p < 0.007). These decreases brought the serum concentrations to about normal levels. However, IFN had varying effects on serum androgen concentrations in the men not in remission: a small decrease, a large increase in one androgen, or no change in serum androgens.

Conclusions—The association between serum androgen levels and the progression or remission of HIV associated KS suggests that androgens affect the development of KS lesions. A clear understanding of the changes in the androgen environment may provide a sound basis for the development of new therapeutic strategies.

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Despite considerable study, the pathophysiology and natural history of AIDS related Kaposi's sarcoma (KS) have not been fully elucidated. The description of a novel human herpes virus[1-3] as the probable cause of KS has focused research on a single aetiology hypothesis for this disease which was expected to lead to readily accessible treatment options. However, a recent survey has indicated that the relative risk for KS remains unchanged in patients treated with antiviral agents such as ganciclovir or foscarnet.[4] Furthermore, the growth characteristics of KS cells cultured in vitro indicate the importance of a number of factors such as interleukin (IL)-6,[5 6] basic fibroblast growth factor (bFGF),[7] oncostatin M,[8] the HIV-1 *tat* protein,[9] and glucocorticoids.[10] A complex, multifactorial model leading to the genesis of KS in HIV infected patients has been postulated.[11] Current treatment, including chemotherapy, radiotherapy, interferon (IFN), and topical treatment, alone or in combination, is usually only palliative.[12 13] However, IFNα can induce complete remission in a well defined subgroup of patients with AIDS related KS.[14]

We have shown that HIV positive men with KS have different steroid hormone profiles, particularly with respect to androgens, compared with HIV positive men without KS having the same CD4 lymphocyte count.[15] High serum androgen concentrations, including dehydroepiandrosterone (DHEA), DHEA sulphate, and testosterone have been observed in patients with KS. Several types of IFNs stimulate cortisol production,[16] but inhibit gonadal steroidogenesis,[17-19] decreasing serum oestradiol and progesterone in women[20] and serum testosterone in men.[21]

We studied 32 men with HIV related KS who had been treated with recombinant IFNα-2a. At the end of IFN treatment the patients were either in complete KS remission (24 men) or not in remission (eight). The serum adrenal (DHEA, DHEA sulphate) and gonadal (androstenedione, testosterone) androgen concentrations and serum sex hormone binding globulin (SHBG) concentrations were determined before, during, and after IFN treatment. The clinical development of KS was correlated with the serum androgen concentrations.

Patients and methods

PATIENTS

All patients were followed up at the Hospital Rothschild, University Paris VI–Medical Centre in Paris, France. Serum from patients with AIDS (as defined by the Centers for Disease Control and Prevention criteria (1987)) and biopsy proven or clinically documented KS, treated mostly between 1986 and 1991 with recombinant IFNα-2a (Hoffmann-LaRoche), were retrospectively tested. IFN treatment was not initiated during an acute infection, or in patients with a past history of opportunistic infections. The drug was given as an intramuscular dose of 9 million units on day 1, followed by 18 million units per day until there was a
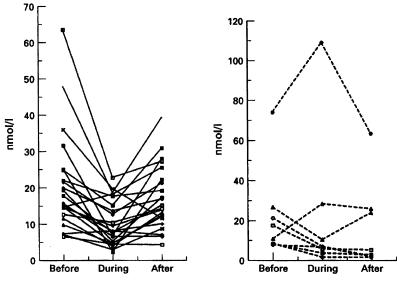
Figure 1   Serum DHEA concentrations determined by radioimmunoassay in HIV positive men with KS before, during, and after treatment with IFNα-2a. Left, patients in complete KS remission (n=24); right, patients not in KS remission (n=8).

radioimmunoassay (RIA) of DHEA (yield 90–95%).

DHEA sulphate DHEA sulphate concentration was determined directly without extraction. Samples were diluted with radioimmunoassay buffer for RIA.

All data were corrected for sample loss during extraction and separation.

RADIOIMMUNOASSAY

Samples of androstenedione, testosterone, DHEA, and DHEA sulphate were assayed using rabbit antisera—anti-androstenedione 7-α-BSA serum and anti-testosterone-7-α-BSA serum from Miles, Yeda Ltd, Israel; anti-DHEA 15-CH$_2$-CO-BSA and anti-DHEA sulphate 7-β-CM-BSA from Biosys, France. The detection limit was 18 pmol/l in all cases.

The tritiated steroids 1,2,6,7[3]H androstenedione (96 Ci/mmol), 1,2,6,7[3]H testosterone (94.1 Ci/mmol), 1,2,6,7[3]H DHEA (86.6 Ci/mmol) and 1,2,6,7[3]H DHEA sulphate (76.8 Ci/mmol) were purchased from the Radiochemical Centre, Amersham. All were 99% pure; purity was checked by thin layer chromatography. Radioactivity was determined on samples dissolved in 4 ml Opti-Fluor (Packard, Chicago, USA) by counting in a Packard 1500 liquid scintillation analyser using the internal standard for quench correction.

SEX HORMONE BINDING GLOBULIN

SHBG concentrations were measured using the DELFIA (Wallac Oy, Turku, Finland) SHBG kit (time-resolved fluoroimmunoassay).

STATISTICS

Data were analysed using non-parametric Wilcoxon and Spearman tests (SPSS 4.0, for Macintosh). Statistical significance was set at p < 0.05.

response, and for at least two months after the maximal response. Complete tumour response was defined as the disappearance of all palpable lesions. All other responses, stabilisation or progressive disease were classified as non-response.

Thirty two male homosexuals with HIV related KS, aged 29 to 55 years (mean 41.7 years) were studied: 24 were in complete KS remission following IFN treatment, and eight were not in remission. Mean duration of treatment was 7.7 months (range 2–18). The mean interval between taking the first serum sample (before) and initiation of treatment was 15 days (range 1–53). The second serum sample (during) was taken mid-treatment ±1 month. The mean interval between the third serum sample (after) and the end of treatment was 44 days (range 20–120). Patients were not treated with ketoconazole or glucocorticoids at any time at this centre before or during treatment, or during follow up.

BLOOD SAMPLES

Blood samples were collected between 0800 and 1000 and allowed to coagulate before separating the serum by centrifugation (1000 *g* for 10 minutes at 4°C). Serum samples were stored at −20°C until assayed.

ANDROGEN EXTRACTION AND CHROMATOGRAPHIC FRACTIONATION

Androstenedione and testosterone Serum samples (0.5 ml) were extracted for 30 minutes with 5 ml organic solvent (ethyl acetate/cyclohexane, 1/1 vol/vol) and the aqueous phase was removed by freezing (−20°C). The organic phase was evaporated to dryness, taken up in 1 ml solvent system I (benzene/ethanol, 95/5 vol/vol) and placed on a Sephadex LH20 microcolumn (0.5×6 cm). Androstenedione and testosterone were eluted with 5.5 ml solvent I (70–95% yield).

Dehydroepiandrosterone Serum samples (0.5 ml) were extracted with organic solvent (ethyl acetate/cyclohexane, 1/1 vol/vol) for direct

Results

ANDROGEN CONCENTRATIONS IN PATIENTS IN COMPLETE KS REMISSION

The serum androgen concentrations of HIV positive men in complete KS remission were significantly lower during treatment with IFN than before treatment. All, or at least three androgens decreased dramatically in these patients. The serum androgen concentrations of these men were higher than those during treatment. The androgens returned to the before treatment concentrations after IFN treatment. All percentages given below are mean values.

DHEA (fig 1)

During treatment There was a 51% decrease from before treatment concentrations (median drop 2.15 nmol/l, p < 0.0001) for DHEA in all patients in complete remission except two in whom it increased by 24% and 9%.

After treatment There was an 82% increase in 20 patients compared with during treatment values.

DHEA sulphate (fig 2)

During treatment There was a 38% decrease from before treatment concentrations (median
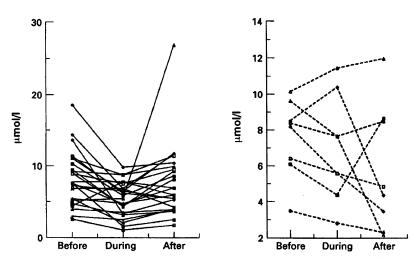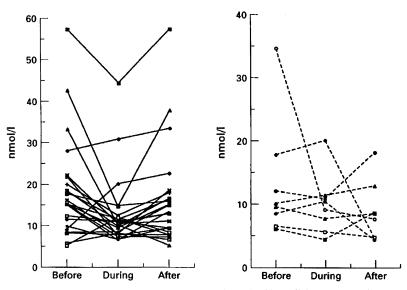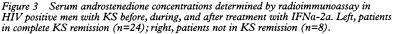
Figure 2   Serum DHEA sulphate concentrations determined by radioimmunoassay in HIV positive men with KS before, during, and after treatment with IFNa-2a. Left, patients in complete KS remission (n=24); right, patients not in KS remission (n=8).

Figure 3   Serum androstenedione concentrations determined by radioimmunoassay in HIV positive men with KS before, during, and after treatment with IFNa-2a. Left, patients in complete KS remission (n=24); right, patients not in KS remission (n=8).
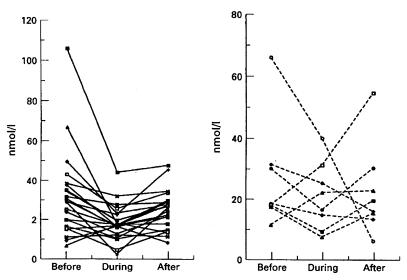

Figure 4   Serum testosterone concentrations determined by radioimmunoassay in HIV positive men with KS before, during, and after treatment with IFNa-2a. Left, patients in complete KS remission (n=24); right, patients not in KS remission (n=8).

drop 0.45, p < 0.002) for DHEA sulphate in all patients in complete remission except three, in whom it increased by 3% in one and did not change in the remaining two patients.

After treatment There was a 68.5% increase in 20 patients compared with during treatment values.

Androstenedione (fig 3)
During treatment There was a 39% decrease from before treatment concentrations (median drop 1.91, p < 0.002) for androstenedione in all patients in complete remission except four, in whom it increased by 2–11%.

After treatment There was a 39% increase in 15 patients compared with during treatment values.

Testosterone (fig 4)
During treatment There was a 44% decrease from before treatment concentrations (median drop 2.91, p < 0.007) for testosterone in all patients in complete remission except one, in whom it increased by 10%.

After treatment There was a 45% increase in 21 patients compared with during treatment values.

PATIENTS NOT IN KS REMISSION
In the patients not in KS remission during treatment with IFN, the serum androgen concentrations increased, remained unchanged or decreased. Large increases of at least one androgen were observed in each patient not in KS remission: DHEA concentration increased by 47% and 166% (fig 1, right); DHEA sulphate increased by 22% and 14% (fig 2, right); androstenedione increased by 13% (fig 3, right); and serum testosterone increased by 72% and 93% (fig 4, right). In the patients not in KS remission we also observed no change in the serum androgen concentrations of at least two androgens. Finally, androgen decreases observed in men not in KS remission were similar to those observed in the patients in complete remission; however, serum concentrations in the patients not in remission were still above the normal concentrations before treatment.

The serum androgen concentrations increased, or remained elevated after IFN treatment.

SEX HORMONE BINDING GLOBULIN
The serum SHBG concentrations were not significantly altered by treatment with IFN in the majority of HIV positive men regardless of KS remission state: no change was seen in 19 of 24 patients in complete remission, and six of eight patients not in remission (table 1). There was a slight, non-significant increase in SHBG concentrations during IFN treatment in both groups compared to pretreatment levels.

Discussion
The results of this retrospective study suggest that the serum concentrations of androgens in HIV positive men with KS are markedly modified by exogenous IFN. The adrenal (DHEA, DHEA sulphate) and gonadal (testo-

Table 1 Serum sex hormone binding globulin concentrations in HIV positive men with Kaposi's sarcoma (KS) before, during, and after treatment with interferon a-2a

Patients	Before	During	After
Complete KS remission	66.6 (9.6)	82.8 (11.5)	68.2 (7.7)
Not in KS remission	89.1 (19.6)	108.0 (22.6)	67.5 (16.0)

Values are mean (SEM) nmol/l.

sterone, androstenedione) androgens are significantly decreased during treatment with IFN. The decrease in androgens appears to be correlated with KS remission. All HIV positive men in complete KS remission had large decreases in serum concentrations of all or at least three androgens during IFN treatment. In contrast, IFN treatment had different effects on the serum androgens in the HIV positive men without KS remission. In these patients the decrease in serum androgen concentrations was in general less than in patients in complete remission and the decrease did not concern all androgens. Indeed, a large increase in serum concentrations of one androgen was observed in each patient.

SHBG concentrations increased, but not significantly, in both groups during treatment with IFN. These increases do not appear to involve the androgens, as there was no significant difference in the SHBG concentrations in the remission versus the non-remission group. This lack of correlation between androgen concentrations and SHBG levels indicates that mechanisms other than SHBG might be involved in androgen variations during IFN treatment.

The decrease in serum testosterone concentrations in response to IFN has been reported previously.[17] Exogenous human leucocyte IFNα decreased serum testosterone concentrations of healthy men, and these concentrations returned to pretreatment levels after cessation of IFN. The decrease in serum testosterone was not accompanied by any consistent change in concentrations of trophic hormones (follicle stimulating hormone and luteinising hormone).[18] This suggests that IFNα interferes with the action of trophic hormones on the target tissue. The inhibitory effect of IFNα on testosterone production was significantly enhanced by adding IFNγ,[19] which also inhibited human chorionic gonadotrophin stimulated testosterone production by Leydig cells.[20] The effect of IFNγ resulted in a decrease in the concentrations of mRNA of two steroidogenic enzymes, P450 cholesterol side chain cleavage enzyme and 17α-hydroxylase/17-20 lyase.[21]

We have, therefore, demonstrated that HIV positive men with KS have higher circulating androgen concentrations than HIV positive men without KS with similar CD4 counts,[15] and that serum androgen concentrations are decreased when IFN is given. This decrease in androgens results in a normalisation of the serum concentrations of these steroids which is associated with remission of KS. This raises the question of the mechanisms by which androgens affect the progression of KS lesions. Further studies on the relationship between androgen concentrations, cytokine profile, T cell proliferation and activity, and a possible

viral aetiology in HIV associated KS should indicate the role of androgens in the interactions leading to this disease. However, the high androgen concentrations in HIV positive men with KS, particularly in the early stages of the disease (with CD4 counts > 500/ml),[15] the changes in concentrations during the development of HIV infection,[22] and their correlation with CD4 cell count[23][24] suggest that these steroids may affect the immune system by causing an abnormal cytokine profile and altering CD8 proliferation and activity. The adrenal androgens are known to enhance the capacity of activated T cells to produce IL-2 and IFNγ. This action may indirectly favour the secretion of type 2 cytokines,[25] such as IL-6, which act as autocrine–paracrine growth factors for AIDS associated KS.[5][26] The gonadal androgen, testosterone, also stimulates suppressor T cell function[27] and induces the expression of transforming growth factor β[28]; this also increases in patients with KS.[29] The association between androgens and the progression or remission of HIV associated KS suggests that a clear understanding of the changes in the androgen environment may provide a sound basis for the development of new therapeutic strategies.

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