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Comparison Between Testosterone Enanthate-Induced Azoospermia and Oligozoospermia in a Male Contraceptive Study. V. Localization of Higher 5α-Reductase Activity to the Reproductive Tract in Oligozoospermic Men Administered Supraphysiological Doses of Testosterone

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ABSTRACT: Sex steroid contraceptive regimens result in incomplete suppression of spermatogenesis in 30–45% of Caucasian men. The basis for this is unclear, but differences in the activity of 5α-reductase (5αR) have been demonstrated. Two isoforms of 5αR have been described: 5αR1 is found in skin, whereas the predominant form in reproductive tissues is 5αR2. To investigate possible contributions of these isoenzymes, we have investigated androgen-dependent changes in seminal plasma androgens (5αR2) and sebum production (5αR1) during administration of a supraphysiological dose (200 mg IM weekly) of testosterone enanthate (TE) to 33 normal men.

Eighteen men rapidly (<20 weeks treatment) became azoospermic, the remainder having a mean sperm density of 2.0 ± 0.6 × 10^6 at that time. The concentrations of testosterone and 3α,17β-androstanediol glucuronide (AdiolG) were lower in seminal plasma than in blood but rose by a similar degree (100%) after 16 weeks TE treatment in both groups. There were no differences in seminal-plasma concentration of testosterone or AdiolG between azoospermic and oligozoospermic responders, either pretreatment or after 16 weeks TE treatment. Although the concentrations of dihydrotestosterone (DHT) were similar in seminal plasma and blood pre- and posttreatment, there was a selective increase in seminal plasma DHT concentration in the oligozoospermic responders from 2.12 ± 0.29 to 2.94 ± 0.33 nmol/L ($P < 0.05$), while there was no significant change in the azoospermic responders (2.18 ± 0.31–2.54 ± 0.27 nmol/L) after 16 weeks of TE treatment. Dihydrotestosterone in seminal plasma is primarily derived from 5αR activity in the epididymis. The concentration of prostaglandin E2 (PGE2) in seminal plasma was unchanged during TE treatment. Sebum excretion was increased during TE treatment, but there were no differences between azoospermic and oligozoospermic responders pretreatment or after 16 weeks TE treatment. These results are consistent with the hypothesis that incomplete suppression of spermatogenesis during TE treatment is associated with a relatively higher 5αR activity in the reproductive tract (epididymis and/or testis) during TE treatment. As the predominant form of 5αR in the reproductive tract is 5αR2 (type 2), we conclude that the increase in activity derives from this form of the enzyme, rather than the type 1 form (5αR1) predominantly found in nongenital skin.

Key words: Male contraception, spermatogenesis, prostaglandin E, seminal plasma, sebum production.

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Administration of supraphysiological doses of testosterone to normal men causes inhibition of gonadotrophin secretion and thus suppression of spermatogenesis. This is currently being investigated as a method of hormonal contraception in men. Although over 98% of men suppress to severe oligozoospermia (<3 × 10^6/ml), azoospermia is achieved in only 55–70% of Caucasian men (World Health Organization, 1990, 1996). The basis for this heterogeneity in response is at present unclear. We have recently reported that there may be an overall increase in 5α-reductase (5αR) activity in those men who remain oligozoospermic during testosterone enanthate (TE) treatment (Anderson et al, 1996). However, the localization of 5αR activity and the relative contributions of reproductive versus nonreproductive tissue to this overall increase is unknown. A more direct assessment of 5αR activity in the male reproductive tract may be provided by the study of its secretory function and the concentration of 5α-reduced androgens therein. The concentration of dihydrotestosterone (DHT) in seminal plasma after vasectomy is reduced to 20–40% of normal, but the con-

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centration of testosterone (T) is unchanged or only slightly reduced (Purvis et al, 1975; Le Lannou et al, 1980; Ying et al, 1983). These findings suggest that most dihydrotestosterone (DHT) in seminal plasma derives from 5αR activity in the testis/epididymis rather than the accessory glands, whereas T in seminal plasma is derived in more equal measure from the testis/epididymis and the accessory glands. The measurement of DHT in seminal plasma may therefore be regarded as a marker for testicular/epididymal 5αR activity, which is known to be hormonally regulated (Robaire et al, 1977; Viger and Robaire, 1991; Silver et al, 1994).

Two isoenzymes of 5αR have been described (Jenkins et al, 1992) with a differential anatomical distribution. Thus, 5αR1 (type 1) is the predominant form in nongenital skin, whereas type 2 (5αR2) predominates in the reproductive tract (Thigpen et al, 1993). In the skin, enzyme activity is mostly localized to the apocrine sweat and sebaceous glands. In patients with hereditary 5αR deficiency, in which there is selective loss of 5αR2 activity, sebum excretion is normal (Imperato-McGinley et al, 1993). Treatment with finasteride, a selective inhibitor of 5αR2 activity, also resulted in no change in sebum excretion in eugonadal men (Imperato-McGinley et al, 1993).

Physiological levels of androgen are required for the normal function of the accessory reproductive glands, including the production in the seminal vesicles of the very high concentrations of prostaglandins (PG) found in seminal plasma, which are predominantly of the E series (PGE) (Skakkebaek et al, 1976; Templeton et al, 1978). Whether the administration of supraphysiological doses of T further increases seminal plasma PG production is at present unknown. 5α-Reductase is present in the seminal vesicle, and as in the prostate, its expression is dependent on androgen (Silver et al, 1994). The function of 5αR and the relative importance of T and DHT in the seminal vesicle are not well defined, but it is probable that it is involved in the control of secretory activity, e.g., seminal fluid and PGs. Thus, seminal vesicle function is abnormal in subjects with 5αR deficiency (Cai et al, 1994), and men treated with finasteride also report reduced ejaculate volumes (Gormley et al, 1992), but there are no data on effects on PG concentration. The possibility that seminal plasma PG concentration may reflect accessory gland 5αR2 activity has therefore not been explored.

We have therefore investigated whether the maintenance of a low rate of spermatogenesis during TE treatment is associated with differences in markers of 5αR activity in the reproductive tract. We have also measured sebum excretion, which may reflect 5αR1 rather than 5αR2 activity. The effect of TE treatment on seminal plasma PG concentrations is also reported.

Subjects and Methods

Subjects and Design

Thirty-three healthy men aged 21–41 years (mean 31 ± 1 SEM) were recruited into a clinical efficacy trial of hormonal male contraception after screening medical examination and biochemical and hematological analyses. All subjects produced two baseline pretreatment semen samples after at least 2 days abstinence with sperm densities of >20 × 10^6/ml and two blood samples with concentrations of gonadotrophins and T within the reference range for adult males. Each subject received 200 mg TE ("Testoviron," Schering AG, Berlin, Germany) IM weekly and were required to use this as their only method of contraception for 12 months once their sperm density had fallen to below 5 × 10^6/ml. This study received the approval of the Lothian Paediatric/Reproductive Medicine Ethics Committee.

Semen samples were analyzed according to the WHO laboratory manual for the examination of human semen and semen–cervical mucus interaction (World Health Organization, 1987) after 3 days of ejaculatory abstinence at 2–4 week intervals. Azoospermia was confirmed by examination of the pellet after centrifugation on three consecutive samples. Subjects were classified into azoospermic and oligozoospermic responders on the basis of sperm density after 20 weeks of TE treatment (Anderson and Wu, 1996). At that time, all men in the azoospermic group had achieved that state, and no spermatozoa were detected in subsequent ejaculates during TE treatment. None of the oligozoospermic responders became azoospermic with up to 40 weeks continued TE treatment.

Measurement of seminal plasma concentrations of T, DHT, 3α,17β-androstanediol glucuronide (AdiolG), and PGE2 were carried out pretreatment and after 16 weeks TE treatment.

Assays

After conventional semen analysis, semen samples were centrifuged at 3,000 rpm for 5 minutes and seminal plasma stored at −20°C. For PG determination, 100 μl seminal plasma was added to 1 ml methyl-oximating buffer before storage. Testosterone was measured as previously described (Corker and Davidson, 1978), with mean recovery of 76%. Dihydrotestosterone was measured using the [^{3}H]T/DHT assay system (Amersham International, Little Chalfont, Buckinghamshire, UK). This involves oxidation of T by permanganate, causing it to lose immunoreactivity to the anti-DHT/T antibody. The efficiency of the oxidation stage in the DHT assay was checked and found to be complete (>99%) in seminal plasma spiked with 100 nmol/L T for each batch of reagents. Recovery was also confirmed by adding known quantities of DHT to samples: mean recovery was 105 ± 7% (n = 8) over the range 50–800 pg DHT added. Intraassay and interassay coefficients of variation were 6.4 and 9.4% for T and 7.5 and 9.9% for DHT at 2.6 nmol/L. Assay sensitivity was 0.3 nmol/L for T and 0.9 nmol/L for DHT.

3α,17β-Androstanediol glucuronide was measured using a commercial kit (Diagnostic Systems Laboratories Inc., Webster, Texas). The antibody used for measurement of AdiolG is directed against 3α-androstanediol 17-glucuronide-7-hemisuccinate and has been shown to give values in good agreement with previous chromatographic methods (Samojlik and Kirschner, un-

Table 1. *Concentrations of T, DHT, and AdiolG in seminal plasma, pretreatment and after 16 weeks TE treatment in all subjects*

	Pretreatment	16 Weeks TE
T	1.28 ± 0.12	2.56 ± 0.32*
DHT	2.39 ± 0.21	2.70 ± 0.23
AdiolG	5.53 ± 1.54	10.12 ± 1.85*

T, testosterone; DHT, dihydrotestosterone; AdiolG, 3α,17β-androstanediol glucuronide; TE, testosterone enanthate.
* $P < 0.001$ versus pretreatment. All values, mean ± SEM, nmol/L.

published data). This antibody has 10.7% crossreactivity with unconjugated Adiol, 5.9% with Adiol-3-glucuronide (which contributes approximately 20% to total serum AdiolG) (Thompson et al, 1991) and <2% with T glucuronide and DHT glucuronide. Validation of the AdiolG assay for use with seminal plasma samples was carried out by demonstrating linearity of dilution and recovery (mean recovery 105 ± 8%, $n = 8$) after spiking of seminal plasma with authentic AdiolG. Intraassay and interassay coefficients of variation were 6.1 and 6.4%. Assay sensitivity was 0.5 nmol/L. Results presented were derived from two assays, and samples from both azoospermic and oligozoospermic responders were included in both to minimize interassay variation.

Prostaglandin E2, was measured by radioimmunoassay (RIA) using an established in-house assay. Because of the wide intersample variation, results are expressed as the mean of two pretreatment samples and the mean of 8- and 16-week treatment samples.

Measurement of Sebum Excretion Rate (SER)

The gravimetric technique of Strauss and Pochi (1961) was used. Measurements were taken before TE treatment was started and repeated after 16 weeks. Measurements were carried out starting at 9:00 AM while the subjects rested semiprone, and the temperature of the room was maintained at 21–23°C. Nonabsorbent tape was used to mask 5 cm^2 areas of the forehead above the eye and the upper back over the blade of the scapula. Both left and right sides of the body were sampled to give bilateral determinations for each subject. Pads of four cigarette papers, prepared by washing in ether, were held in place over each of the four masked areas with bandaging and adhesive tape, using a gauze swab as a pressure pad for 15 minutes each and discarded. This was repeated. A third pad was then applied to each area and left in place for 3 hours. At the end of the collection period, the sebum-loaded pads were removed. The weight of sebum was determined after extraction into ether, followed by drying. In each batch, blanks were included consisting of pads prewashed as in the preparation stage but not applied to the skin and were extracted in the same fashion as samples. Results were calculated as SER in mg/3 hours/10 cm^2, the sum of left- and right-sided determinations.

To assess the reproducibility of the method, samples from the right and left of the body were compared before being combined to give the final value. The correlation coefficient between left and right was 0.91 pretreatment and 0.82 during TE treatment.

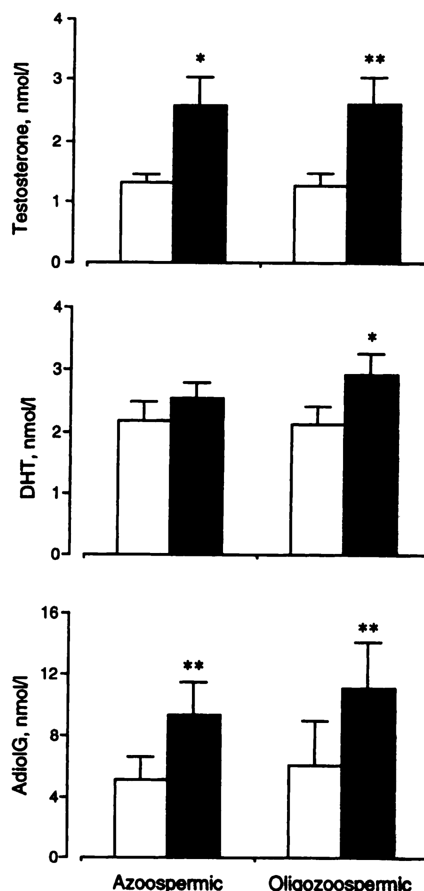

FIG. 1. Seminal plasma concentrations of **(a)** testosterone, **(b)** dihydrotestosterone, and **(c)** 3α,17β-androstanediol glucuronide (AdiolG) pretreatment and after 16 weeks of testosterone enanthate (TE) treatment in azoospermic (n = 18, open columns) and oligozoospermic responders (n = 15, filled columns). *$P < 0.05$, **$P < 0.01$ versus pretreatment.

Statistical Analysis

Data are presented as mean ± SEM. Results were compared by Student's *t*-test using paired values where appropriate after logarithmic transformation to correct for non-Gaussian distribution.

Results

Androgens

Seminal plasma concentrations of testosterone, DHT and AdiolG, pretreatment and after 16 weeks TE in all subjects, are shown in Table 1. There were significant increases in seminal plasma concentrations of T and AdiolG with TE treatment, but there was no significant increase in DHT. However, a different pattern emerged when subjects were divided into azoospermic and oligozoospermic responders (Fig. 1). In the oligozoospermic group, DHT was increased from 2.12 ± 0.29 to 2.94 ± 0.33 nmol/L ($P < 0.05$) after the 16-week TE treatment, while there was no significant change in the azoospermic group (2.18

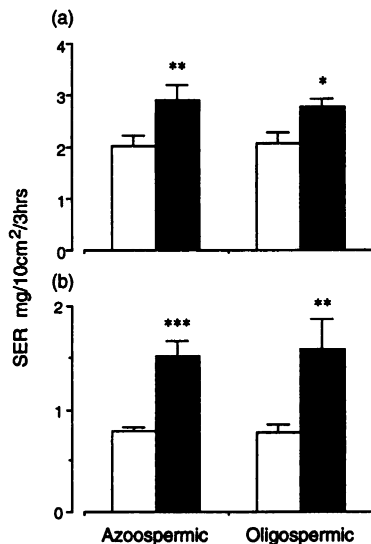

FIG. 2. Sebum excretion rate in azoospermic and oligozoospermic responders, pretreatment and after 16 weeks of testosterone enanthate (TE) treatment from **(a)** forehead and **(b)** upper back. Open columns, baseline; filled columns, after 16 weeks of TE treatment. Mean ± SEM. $^*P < 0.02$, $^{**}P < 0.01$; $^{***}P < 0.001$ versus pretreatment.

± 0.31 nmol/L pretreatment, 2.54 ± 0.27 nmol/L after the 16-week TE treatment). Testosterone concentrations in seminal plasma were increased in both groups of men, from 1.31 ± 0.13 nmol/L to 2.55 ± 0.47 nmol/L in the azoospermic group ($P < 0.02$) and from 1.25 ± 0.21 nmol/L to 2.58 ± 0.46 nmol/L in the oligozoospermic group ($P < 0.01$). Seminal plasma concentrations of AdiolG were also increased in both groups. In the azoospermic group, AdiolG concentrations were increased from 5.1 ± 1.5 to 9.3 ± 2.3 nmol/L ($P < 0.005$) and in the oligozoospermic group from 6.0 ± 3.0 to 11.1 ± 3.1 nmol/L ($P < 0.001$). There were no differences in concentration of any of the three steroids between the two groups, pretreatment or after the 16-week TE treatment.

Sebum Excretion Rate

Mean pretreatment SER from forehead was 2.02 ± 0.02 mg/3 hours/10 cm^2 in azoospermic responders and 2.06 ± 0.22 mg/3 hours/10 cm^2 in the oligozoospermic responders. Sebum excretion rate was consistently less from upperback skin, being 0.78 ± 0.04 mg/3 hours/10 cm^2 in the azoospermic responders and 0.77 ± 0.08 mg/3 hours/ 10 cm^2 in the oligozoospermic responders. This difference between forehead and back was significant for both groups ($P < 0.001$). There was no difference in SER of either area between the two groups pretreatment.

After 16 weeks of TE treatment, SER was increased in all men from both the forehead and the back (Fig. 2). In the azoospermic responders, forehead SER had increased to 2.90 ± 0.31 mg/3 hours/10 cm^2 ($P < 0.01$) and from the upper back to 1.51 ± 0.15 mg/3 hours/10 cm^2 ($P < 0.001$). In the oligozoospermic responders, forehead SER had increased to 2.77 ± 0.15 mg/3 hours/10 cm^2 ($P < 0.02$) and from the upper back to 1.58 ± 0.29 mg/3 hours/ 10 cm^2 ($P < 0.01$). There were again no differences between the two groups.

Table 2. *Seminal plasma PGE2 concentrations in azoospermic and oligozoospermic responders, pretreatment and during TE treatment*

	PGE2 concentration (μg/ml)	
	Pretreatment	During TE treatment
Azoospermic responders	59 ± 9	55 ± 12
Oligozoospermic responders	53 ± 8	50 ± 8

PGE2, prostaglandin E2; TE, testosterone enanthate.

PGE2

There was a wide intersample range of seminal plasma PGE2 concentrations, thus results are presented as the mean of two pretreatment samples and the mean of samples after 8 and 16 weeks TE treatment. There was no significant difference between PG concentrations at 8 and 16 weeks. Seminal plasma concentration of PGE2 was 56 ± 4 μg/ml pretreatment and was 53 ± 8 μg/ml during TE treatment. There were no differences when subjects were divided into azoospermic and oligozoospermic responders (Table 2).

Discussion

The concentration of DHT in seminal plasma is similar to that in blood (Purvis et al, 1975) and appears to be largely of testicular/epididymal origin. Thus, it is reduced after vasectomy and in obstructive azoospermia to approximately 25% of normal (Purvis et al, 1975; Le Lannou et al, 1980; Ying et al, 1983). Testosterone is converted to DHT by the 5αR, and two isoenzymes have been identified (Jenkins et al, 1992). The type 2 isoenzyme is crucial for fetal development of the prostate and external genitalia and is present in the adult human seminal vesicle and epididymis (Thigpen et al, 1993) where its continued expression is dependent on circulating androgens (Silver et al, 1994). 5α-Reductase activity in the rat epididymis is also positively regulated by androgens (Robaire et al, 1977), and physical connection to the testis appears necessary for normal activity (Robaire, 1979; Viger and Robaire, 1991). Dihydrotestosterone concentration in seminal plasma may, therefore, be a marker of epididymal function (Zalata et al, 1995) and, in particular, 5αR2 activity. Sperm density has also been shown to correlate with seminal plasma DHT (Paulson et al, 1986; Zalata et al, 1995). Pretreatment concentrations of DHT

reported here are similar to those in previous reports. After the 16-week TE treatment, the concentration of DHT in seminal plasma was significantly increased only in the oligozoospermic, not azoospermic responders. This suggests that epididymal 5αR activity may be greater in those men able to maintain spermatogenesis, albeit at a severely reduced rate during gonadotropin suppression. This is consistent with the hypothesis that the persistence of spermatogenesis is associated with relatively higher overall 5αR activity (Anderson et al, 1996). The present results therefore extend our previous findings, suggesting that the increase in 5αR during TE treatment may be, at least in part, localized to the reproductive tract and may therefore reflect activity of the type 2 isoenzyme. During treatment with supraphysiological doses of T, intratesticular T levels are low (Morse et al, 1973), secondary to suppression of LH secretion. Under experimentally induced intratesticular T depletion, it has recently been shown that DHT plays an important role in the restoration of spermatogenesis in the rat (O'Donnell et al, 1996). However, it is also possible that an increased 5αR activity in the epididymis is caused by the low level of sperm production (or other testicular factors delivered to the epididymis).

Testosterone and AdiolG in seminal plasma showed a different pattern to that of DHT. The concentration of T in seminal plasma was much lower than in blood, as previously reported (Paulson et al, 1986; Zalata et al, 1995) but was nevertheless increased by TE treatment in both azoospermic and oligozoospermic responders. The proportional increase was similar to that of T in peripheral plasma (Anderson and Wu, 1996). The pretreatment concentration of AdiolG in seminal plasma was 5.5 nmol/L, approximately 25% of that in serum, similar to the concentration found in the only previous report of AdiolG in seminal plasma (Paulson et al, 1986). 3α,17β-Androstanedial glucuronide in plasma is reduced by adrenalectomy and increased by adrenal stimulation, indicating a significant adrenal contribution as well as that derived from the testis via peripheral conversion of T (Deslypere et al, 1982). The origin of AdiolG in seminal plasma is, however, unknown. Testosterone and AdiolG seminal plasma followed a similar pattern: their concentrations in seminal plasma are considerably lower than in the peripheral circulation, and both showed a proportionally similar increase to peripheral T and AdiolG levels following TE treatment. This may be taken to indicate that these two androgens in seminal plasma are predominantly derived from the peripheral circulation. In contrast, DHT is present in similar amounts in the peripheral circulation and seminal plasma, and the increases in DHT are much smaller (in fact, there was no change in seminal plasma DHT concentration in the azoospermic group). This suggests that local mechanisms in the testis/epididymis may play a key role in regulating the ambient concentration of DHT in the reproductive tract and that 5αR2 activity is involved in this role. There may also be a prostatic contribution.

Despite the high concentrations of PGs in seminal plasma, little is known about their control. Androgens appear to be required for PG production (Skakkebaek et al, 1976), but the results presented here suggest that androgen-stimulated seminal plasma PGE2 secretion may be maximal at physiological T concentrations. The relative importance of T versus DHT in seminal vesicle function and PGE secretion is unknown. However, the absence of any changes in PGE during TE administration may suggest that 5αR2 activity in the seminal vesicle is unaltered. This would further localize the increase of DHT in seminal plasma to the epididymis/testis. Testosterone enanthate, in combination with medroxyprogesterone acetate, was found to have no effect on the concentration of several steroids in seminal plasma, but an increase in the concentration of PGE was detected (Hedman et al, 1988). Seminal plasma PG secretion may therefore be controlled by other, nonandrogenic factors.

The skin also contains high 5αR activity (Hay and Hodgins, 1978), which is of the type 1 isoenzyme (Thigpen et al, 1993). Thus, although sebum excretion was undetectable in subjects with androgen insensitivity, it was normal in subjects with 5αR2 deficiency and unchanged in eugonadal men following finasteride treatment (Imperato-McGinley et al, 1993). Testosterone was originally reported to have no effect on SER in adult men (Strauss et al, 1962), although the administration of methyltestosterone to prepubertal boys or eunuchs stimulated SER and increased the size of the sebaceous glands. This was attributed to sebum secretion being already maximally stimulated in adult men (Strauss et al, 1962; Pochi and Strauss, 1974). More recently, it has been shown that anabolic steroids increase sebum secretion and the size of sebaceous glands in athletes (Király et al, 1987a,b), although the multiplicity of steroid preparations taken simultaneously and the variations in the self-administered doses make the androgenic stimulus in those studies difficult to quantify. Our results show an increase in SER during TE treatment, from both forehead and upper back. There were no differences in SER between azoospermic and oligozoospermic responders either pretreatment or during TE treatment. These results do not, therefore, support an increase in skin 5αR1 as being responsible for the increase in 5αR activity as we have previously reported (Anderson et al, 1996).

In conclusion, these data suggest a selective increase in 5αR2 activity in the reproductive tract induced by exposure to supraphysiological doses of exogenous T in those men maintaining a low rate of spermatogenesis. This may reflect an adaptive effect within the testis. The relatively greater availability of DHT in the testis may

underlie the maintenence of a minor degree of spermatogenesis in those 30–40% of men who do not rapidly develop azoospermia despite the suppression of gonadotrophin secretion.

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