ORIGINAL ARTICLE

A New Strategy for the Prevention of Keratoconus Progression?

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Abstract

Purpose: Keratoconus may be associated with imbalances of sex hormones such as reduced testosterone levels in affected males. In this study, an evolutionary and natural mechanism to increase testosterone levels in males via exposure to the synthetic female pheromone fortidin was evaluated.

Materials and methods: In a precursor study $n = 16$ healthy young males were exposed to Placebo, 2 mg fortidin (5 mL of a 0.04% fortidin solution), and 4 mg fortidin (5 mL of a 0.08% fortidin solution). The testosterone levels were measured before and after the exposure. The pre-exposure testosterone levels were measured three times and the postexposure testosterone level only once in the same $n = 16$ individuals. The $n = 16$ mean and standard deviation of the $n = 48$ pre-exposure measurements in comparison to the $n = 16$ post-exposure data were statistically analyzed.

Results: The exposure to 4 mg fortidin (5 mL of a 0.08% fortidin solution) increases the measured testosterone level by 28.8%. This increase in testosterone level is statistically significant ($p < 0.05$). The increase of the testosterone level by the lower concentration was not statistically significant.

Conclusion: The exposure of young healthy men to 4 mg fortidin (5 mL solution containing 0.08% fortidin) results in a significant increase in their testosterone levels. This mechanism may be important in the future for the prevention and/or treatment of keratoconus. Further studies are in progress.

Keywords: Fortidin, Keratoconus, Testosterone.

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Introduction

The onset of keratoconus often occurs during puberty and further progresses during adulthood.[1,2] Keratoconus is characterized by a weakening of the cornea caused by destruction of the collagen framework.[3] Among other factors, hormonal imbalances have been discussed to be relevant in this process.[4] In particular sex hormones and their precursors seem to play an important role.[5] A recent study has shown significantly lower testosterone levels in male keratoconus patients compared to non-keratoconus males.[6] Here a method to increase testosterone levels in males without hormone substitution is presented. This may perhaps help in the future to prevent keratoconus from break-out or from progression at least in males. The background is an evolutionary developed, natural process where a certain composition of short-chain fatty acids produced by females act as a pheromone which increases the testosterone level of the exposed male.[7] The testosterone levels in males before and after exposure to a synthetic copy of the active female pheromone as a continuation of a precursor study were analyzed.[8] Since there exists no name for the synthetic copy of the active human female pheromone so far, we refer to it in the following as fortidin.

Materials and Methods

fortidin is the synthetic copy of the active human female pheromone consisting of a certain composition of short-chain fatty acids. Data from a precursor study performed at the Institute of Sport Science at the University of Vienna were used for a detailed mathematical analysis.[8] Sixteen healthy male students were exposed to fortidin in three identical sessions always on the same day-time and with 7 days interval between the sessions. In each session, the saliva testosterone before and after exposure was measured. The saliva testosterone is a measure of free testosterone—the active testosterone molecules which is not linked to serum proteins. In each session, the sports students were exposed to a different concentration of fortidin. In the first session, a placebo was applied. In the second session, the students were exposed to a 0.04% concentration of a 5 mL fortidin solution (2 mg) and to a 0.08% concentration of a 5 mL fortidin solution (4 mg) in the third session. In the precursor study, the entire procedure to generate the testosterone data was performed correctly, while the statistical analysis of that data was not performed adequately to get the right information.[8] Therefore, an advanced statistical analysis of the testosterone data of the precursor study was

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performed. The precursor study compared only 16 pre- to 16 post-exposition data in simple statistics without collecting the entire 48 pre-exposition data as the right pre-exposure baseline.[8] To collect the 3 times 16 pre-exposition data to the correct 48 pre-exposition baselines (mean and standard deviation), the following mathematical process was applied:

$$t = (n_1 \cdot t_1 + n_2 \cdot t_2 + n_3 \cdot t_3)/(n_1 + n_2 + n_3) = (t_1 + t_2 + t_3)/3$$

and

$$tsd^2 = [s_1^2 \cdot (n_1 - 1) + s_2^2 \cdot (n_2 - 1) + s_3^2 \cdot (n_3 - 1)]/(n_1 + n_2 + n_3 - 1)$$
$$= 15 \cdot [s_1^v + s_2^2 + s_3^2]/47$$

with

t	testosterone level baseline as mean of the $n = 48$ pre-exposed testosterone levels
n_1, n_2, n_3	number of cases in the first, second and third session ($n_1 = n_2 = n_3 = 16$)
t_1, t_2, t_3	mean of pre-exposition testosterone level in each of the three sessions
tsd	standard-deviation of the $n = 48$ pre-exposed testosterone level baseline
s_1, s_2, s_3	standard deviation of the pre-exposition testosterone level in each session

This calculation of the post-collection pre-exposure standard deviation (SD) assumes that the sum of the 3 variances of the (n = 16) individual pre-exposure measurements correspond to 3-times the variance of the (n = 48) collected pre-exposure measurements. A worst case estimation for the pre-exposure standard deviation (TSD) after collection (n = 48) can be obtained from the values mean – SD and mean + SD of each of the 3 pre-exposure measurements as the largest difference of these 6 values from the post-collection pre-exposure mean.

Since the data showed a Gaussian distribution around the mean values in each session,[8] a t-test to compare the post-exposure testosterone level of each session (n = 16) with the baseline pre-exposure testosterone level t (n = 48) was performed.

The related t-test statistics for the comparison of the collected pre-exposure (n = 48) with the post-exposure (n = 16) data revealed $p = 0.011$ and $p = 0.013$ for the worst case calculation of the 4 mg (0.08%) fortidin exposure group. The limit where the significance would be lost would be at an unrealistically high pre-exposure standard deviation of more than 150 pg/mL.

Results

The post-exposure testosterone levels (n = 16) as well as pre-exposure testosterone levels before (n = 16) and after (n = 48) collection are shown in Table 1.

In every session [Placebo, 0.04% (2 mg) and 0.08% (4 mg)] when comparing n = 16 pre-exposure to n = 16 post-exposure an increase of testosterone after exposure to fortidin can be observed.[8] However, when comparing only the single (n = 16) pre-exposure groups as a fraction of the right n = 48 pre-exposure baseline to the (n = 16) post-exposure groups, as in the precursor study, no statistically significance on a $p = 0.05$ level can be detected.

It was mentioned that the lack of statistical significance in the precursor study may be the result of the small groups of n = 16 only used for both, the pre-exposure and the post-exposure group.[8]

Table 1: Saliva testosterone levels (pg/mL)

	Pre-exposure		*Post-exposure*	
	Mean	*Standard deviation*	*Mean*	*Standard deviation*
Placebo (n = 16)	168.0	63.2	179.9	82.2
0.04% Fortidin concentration (n = 16)	170.3	69.3	192.9	51.3
0.08% Fortidin concentration (n = 16)	185.3	59.0	224.7	73.8
Pre-exposure collection (n = 48)	174.53	62.61 (calculated) 73.53 (worst case)		

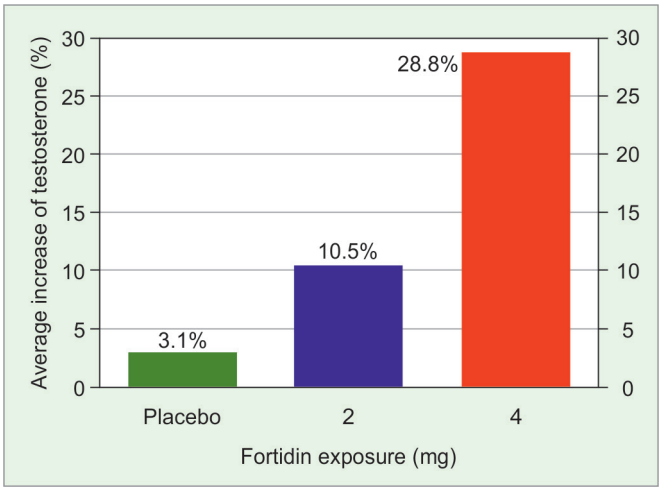

Fig 1: Increase in testosterone in % after exposure to a 5 mL solution containing placebo, 0.04% (2 mg) and 0.08% (4 mg) fortidin, respectively.

Relating the post-exposure testosterone levels to the right n = 48 pre-exposure baseline at 174.53 pg/mL as performed in this study also neither the placebo group nor the 0.04% group showed a statistically significant difference on a $p = 0.05$ level. However, the 0.08% group yielded $p = 0.011$ (worst case $p = 0.013$), and therefore the exposure to a 4 mg fortidin (0.08%) results in a statistically significant increase of the mean of free testosterone. The mean increase of testosterone by exposure to fortidin based on the n = 48 pre-exposure baseline is shown in Figure 1.

The data show that the increase in testosterone level depends on the amount of fortidin to which the males were exposed. The higher the amount of fortidin to which males were exposed the higher the increase in their testosterone level. The highest average increase of free testosterone was 28.8% at the highest applied fortidin amount of 4 mg (0.08%), followed by an increase of 10.5% for the amount of 2 mg (0.04%) and a 3.1% increase only for exposure to nothing (Placebo).

Discussion

In recent years an increasing number of studies appeared showing a relationship between keratoconus and sex hormones or their precursors. For instance dehydroepiandrosterone sulfate (DHEA-S) levels were found to be increased in keratoconus while estrone levels are decreased in keratoconus.[4] It seems that altered

hormone levels modulate metabolism, cytokine, and growth factor expression leading to increased severity of keratoconus.[5] DHEA can bind weakly to sex hormone receptors and it can reduce the testosterone level.[4,5] A recent study showed a reduced testosterone level in male keratoconus patients.[6] It is in agreement with the finding of an increased DHEA level in keratoconus.[4] The fact that testosterone levels are reduced in male keratoconus patients seems a priori reasonable because keratoconus is a disease that is characterized by a weakened (corneal) tissue while a characteristic feature of testosterone is the strengthening of tissue.

On the other side exposition of males to pheromones produced by females during the ovulation phase of the female cycle can increase the testosterone level of the exposed male.[7] These increased testosterone levels make men more susceptible to female attraction. This is an evolutionary developed process to guarantee the reproduction of species and it is widespread in nature.[9] This knowledge is even used to protect vineyards from pests and to reduce pig aggression in farming.[10–12]

In this study, the data of a precursor study that measured the testosterone levels of males before and after exposure to a synthetic copy of the active human female pheromone were analyzed.[8]

Figure 1 and Table 1 show increasing testosterone levels in males after exposure to increasing fortidin levels. Exposure to 4 mg fortidin (0.08%) shows a significant increase of mean free testosterone by 28.8%. The 4 mg fortidin, therefore, seems to be already above the threshold which causes a significant increase in the testosterone level after exposure, while 2 mg is obviously still below that threshold level. This means that the threshold level for a significant increase in testosterone by exposure to fortidin must range between 2 mg (0.04%) and 4 mg (0.08%).

It is also noteworthy that fortidin has a very intensive and characteristic smell and taste which can be recognized even far below a 0.01% concentration. Therefore, taking a placebo into a study may be reasonable if the object of investigation is a pharmaceutical product that is administered in tablet form of identical appearance and taste. However, if fortidin as the object of investigation can easily be distinguished by the test person via smell or taste from a placebo, a placebo control is not applicable. Furthermore, it is obvious from Table 1 that the placebo post-exposure testosterone level is clearly within the normal variation of pre-exposure testosterone levels.

Some 98% of serum testosterone is linked to serum proteins which seem to constitute a reservoir for a quick increase of free testosterone. Free testosterone, which is not linked to proteins is the active testosterone that causes the characteristic biological and medical changes such as virility, muscle growth, alpha-male behavior, endurance, sexual activity, libido, spermiogenesis, EPO increase, etc. Free testosterone only counts for some 2% of the entire testosterone. It can be supposed that exposure to fortidin increases the free testosterone level by reducing the binding capacity of serum proteins to testosterone and thus shifting a part of the protein-linked testosterone pool into the free testosterone pool. A particular advantage of this study is the use of the independent testosterone level data of the precursor study as input into this study which guarantees maximum reliability and objectiveness.

So far only surgical treatment options are available for the treatment of keratoconus against the progression of the disease.[13–16] It is not clear in the moment whether or to what extent exposure to fortidin could be a non-surgical option in the future to prevent humans from keratoconus break-out or from keratoconus progression. Further studies are required to clarify these questions.

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