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Research Article

A Possible New Strategy for Non- Surgical Treatment of Keratoconus

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Abstract

The onset of keratoconus often occurs during puberty and further progresses during adulthood. Keratoconus is characterized by a weakening of the cornea caused by a destruction of the collagen framework. Among other factors hormonal imbalances have been discussed to be relavant in this process. In particular sex hormones and its precursers seem to play an important role. A recent study has shown significantly lower testosterone levels in male keratoconus patients compared to non-keratoconus males. In this study I am presenting a method to increase testosterone levels without hormone substitution. This may perhaps help in the future to prevent keratoconus from break-out or from progression at least in male.

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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 a destruction of the collagen framework [3]. Among other factors hormonal imbalances have been discussed to be relavant in this process [4]. In particular sex hormones and its precursers 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]. In this study I am presenting a method to increase testosterone levels without hormone substitution. This may perhaps help in the future to prevent keratoconus from break-out or from progression at least in male. The background is an evolutionary 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 involved short chain fatty acids are common components of food and approved by the EU as food additives. The testosterone levels in males before and after exposure to a synthetic copy of the active female pheromone (Fortidine) as a continuation of a precursor study [8], were analyzed and treatment of keratoconus with Fortidine under different conditions of use are evaluated.

MATERIAL AND METHODS

Fortidine is the synthetic copy of the active human female pheromone consisting of a certain composition of short-chain fatty acids. In this study I have used data from a precursor study performed at the Instutute of Sport Science at the University of Vienna [8], for a detailed analysis and evaluation of a possible application of Fortidine in the treatment of keratoconus. In the precursor study the same sixteen healthy male students were exposed to Fortidin in three identical sessions always at the same day-time and with seven days intervall between the sessions. In each session the saliva testosterone before and after exposure was measured. The saliva testosterone is a measure of the free testosterone - the active testosterone molecules which is not linked to serum proteins. In each session the sport 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 Fortidine solution (2 mg) and to a 0.08% concentration of a 5ml Fortidine solution (4 mg) in the third session. In the precursor study [8], 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 informations [8].Therefore, in a first step, I have analysed and re-evaluated the available data of the precursor study to get a better and more reliable statistical information about the effect of Fortidin on male testosterone levels. Then I have analyzed a treatment option of keratoconus by means of an oral application. The precursor study [8], compared only 16 pre- to 16 post-exposition data in a simple statistics. I collected the entire 48 pre-exposition data, which were obtained under exactly the same consitions (same persons, same day-time, 1 week intervall) as the right pre-

exposure baseline. To collect the 3 times 16 pre-exposition data to the correct 48 pre-exposition baseline (mean and standard deviation), the following mathematical process was appiled:

$$t = (n1.t1 + n2.t2 + n3.t3) / (n1 + n2 + n3) = (t1 + t2 + t3) / 3$$

and

$$tsd2 = [s12.(n1 - 1) + s22.(n2 - 1) + s32.(n3 - 1)] / (n1 + n2 + n3 -1) = 15 . [s12 + s22 + s32] / 47$$

with

t: testosterone level baseline as mean of the n = 48 pre-exposed testosterone levels

n1, n2, n3: number of cases in the first, second and third session (n1 = n2 = n3 = 16) t1, t2, t3 mean of pre-exposition testosterone level in each of the 3 sessions

tsd: standard-deviation of the n = 48 pre-exposed testosterone level baseline s1, s2, s3 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 preexposure 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], I have performed 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).

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.

The experiments in the precursor study were performed by means of an orthonasal application of Fortidine. The orthonasal application of synthetic replica of the pheromone outside a precisely defined experimental setting may be difficult because the effect depends strongly on the right compostition of the constituents in the nasal cavity. These constituents have, however, different vapor pressures. Evaporation of the constituting molecules out of a liquid reservoir, i.e. by a drink via a retronasal pathway, would therefore be a preferable type of clinical application. The solution at the oral mucosa would then act as a liquid reservoir.

I have therefore studied how many milliliter has a sip of such a drink and which concentration of Fortidine in such a drink is required for retronasal application to achieve the same concentration in the nasal cavity as via the orthonasal application. A baverage on a black tea basis was used as a substrate in which the synthetic female pheromone Fortidin was diluted to a 0.08% solution. This 0.08% Fortitin baverage was bottled in 250 ml portions. The average sip from both, the bottle and the glas, was measured by weighing container after every sip. The amount of Fortidin to which the target tissue was exposed after every sip was calculated and compared to previous experiments, which documented the dose dependent testosterone response. The amount of Fortidin reaching the nasal cavity via the retronasal pathway was related to the application via the orthonasal pathway.

RESULTS

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

In every session (Placebo, 2mg (0.04%) and 4mg (0.08%)) when comparing n = 16 pre- exposure to n = 16 post-exposure an increasing testosterone level with increasing exposure to Fortidin can be observed. However, the statistical analysis of these n = 16 groups revealed no statistical significance on a p = 0.05 level in any of the sessions [8].

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]. 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 2mg (0.04%) group showed a statistical significant difference on a p = 0.05 level. However, the 4mg exposure (0.08%) yielded p = 0.007 and therefore the exposure to the 0.08% Fortidin concentration (4mg) 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 average increase of the testosterone level depends on the Fortidin concentration to which the males were exposed. The higher the Fortidin concentration the higher the increase of testosterone. The highest average increase of free testosterone was 28.8% at the highest applied Fortidin concentration of 0.08% (4mg), followed by an increase of 10.5% for the 0.04% concentration (2mg) and 3.1% increase only for exposure to nothing (Placebo).

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
2 mg Fortidine (n = 16)	170,3	69,3	192,9	51,3
4 mg Fortidine (n = 16)	185,3	59,0	224,7	73,8
Pre-exposure collection (n = 48)	174,53	62,61		

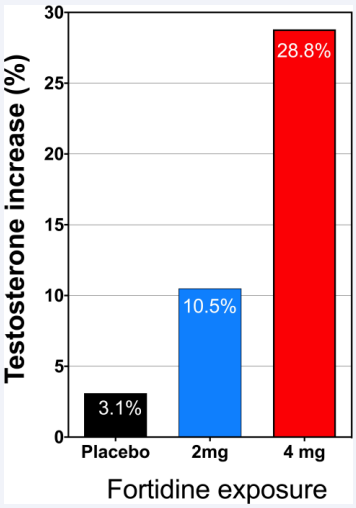

Figure 1 Figure shows the average increase of Testosterone for different Fortidine concentrations.

The sip experiments showed that one sip from the bottle ranges somewhere between 17g and 30g, with some 25g (25 ml) in average. Therefore, one bottle of 250 ml is good for 10 sips. The average sip from a glass was 30g (30 ml) which means that a bottle is good for some 8 sips, when the drink is emptied into a glass before drinking it.

Table 2 shows the related Fortidin exposure, which is some 20 mg per sip when the baverage is drunk from the bottle and some 24 mg per sip when the baverage is drunk from the glass. The total amount of Fortidin to which the body is exposed when drinking the whole bottle is 200 mg.

Table 2: 0.08% Fortidine Baverage

	sip volume (g)	Fortidine content (mg)
Bottle sip	25	20
Glass sip	30	24
Entire bottle	250	200

DISCUSSION

In recent years an increasing number of studies appeared showing a relation 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 which 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 pest and to reduce pig aggression in farming [10-12].

In this study I analysed the data of a precursor study which measured the testosterone levels of males before and after exposure to a synthetic copy of the active human female pheromone [8], and evaluated an oral application of this substance (Fortidine) for the treatment of keratoconus.

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

Considering the retronasal application via a baverage with a 0.08% Foridine concentration the oral cavity is exposed to some 20mg to 24mg of Fortidin, which is 5 to 6 times the value, which causes a significant testosterone boost in male by orthonasal exposure. The perceived flavour intensity after exposure to a short chain fatty acid via the retronasal pathway does not significantly differ from the orthonasal pathway [13]. In general one may expect the retronasal threshold to be around twice (2.25) [14], the orthonasal threshold. This would result in a threshold of retronasal exposure of 9mg Fortidin to cause a significant testosterone increase. Even if one assumes that the retronasal pathway requires twice or even 3 times

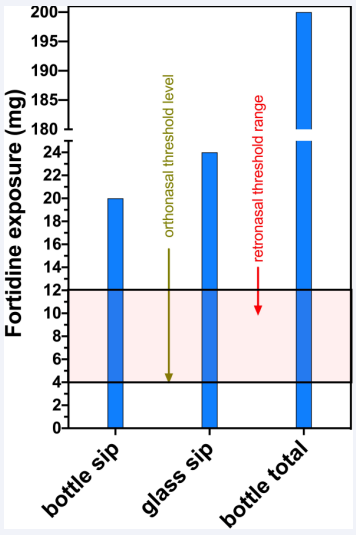

Figure 2 Exposure and threshold values for different application methods.

the concentration to achive the same intensity perception, the number of stimulating molecules reaching the olfactory system from one sip of the 0.08% Fortidin beverage is still far above the threshold for a significant testosterone increase (Figure 2). 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 placebo into a study may be reasonable if the object of investigation is a pharmaceutical product, which is administered in tablet form of identical appearence 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. The 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 behaviour, endurance, sexual activity, libido, spermiogenesis, EPO increase, etc. Free testosterone only counts for some 2% of the entire testosterone. It can be supposed that the 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.

Since Fortidin distributes across the oral and throat mucosa during drinking which is then more or less constantly releasing the constituents in the right composition according to their vapor pressure, one can achive an effective atmosphere in the nasopharynx for testosterone boosting. The presented peroral delivery of Fortidin via a baverage composition can therefore considered a simple, practical and effective method to increase testosterone levels in men.

A particular advantage of this study is the use of the testosterone level data of the independent precurser study as input into this study which guarantees a maximum of reliability and objectiveness.

So far only surgical treatment options are available for the treatment of keratoconus [15,16]. It is not clear in the moment whether or to what extend the 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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