Agents and Actions, vol. 22, 3/4 (1987)

0065-4299/87/040209-05 $ 1.50+0.20/0

The influence of tritoqualin (inhibostamin®) on the plasma histamine level and its biorhythmic variations

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Introduction

Tritoqualin (2-N-Methyl-6,7-methylendioxy-8-methoxy-1-(4′,5′,6′-triethoxy-7′-aminophthalidyl)-1,2,3,4-tetrahydroisochinolin) has been used in the therapy of pruritus [1], hay fever and other allergic diseases of the upper respiratory tract [2, 3, 4] for many years. There is evidence proven that tritoqualin displays protective properties against anaphylactic asthma of the Guinea pig and reduced gastric secretion in rats [5]. Several investigators were able to demonstrate that acute inflammatory URT-affections go parallel with an increase of tissue histamine concentrations in the bronchial mucosa, correlating with a high number of mastcells [6, 7]. Additionally, it could be shown that Tritoqualin is able to decrease gastric mucosal tissue histamine levels in patients suffering from food allergy after provocation with the specific allergens [8]. Tritoqualin seems to exert an unspecific broncholytic activity apart from its postulated action as an inhibitor of histidine decarboxylase (HDC) [9]. It's histamine decarboxylase inhibiting activity seems to be weak [15].

It is documented in literature that histamine plays an important role in the provocation of bronchospasm in asthmatic patients based on the observation that histamine levels are increased during early morning hours in these patients [10]. It is reported that elevated plasma histamine levels during acute periods in patients suffering from seasonal allergic rhinitis are paralleling clinical symptoms whereas histamine levels turned out to be low in the asymptomatic intervals [11]. Recent communications describe biorhythmic variations of plasma histamine concentrations in healthy volunteers [12]. These findings prompted us to investigate the influence of tritoqualine on plasma histamine levels and their biorhythmic changes in healthy volunteers.

Methods

12 volunteers (6 males and 6 females) aging from 23 to 31 years participated in the study. They were divided into two groups by random for reasons of practicability and blood samples were drawn at the following times of the day:

Group I: 7, 9, 11, 12, 13, 15, 17, 18, 19, 21, 23, 1, 3, 5, 7.

From 1 o'clock until 7 o'clock all volunteers were asleep.

Group II: 7, 8, 10, 12, 13, 14, 16, 18, 19, 20, 22, 24, 2, 4, 6.

Blood was sampled from sleeping volunteers between 24 and 6 o'clock.

They were informed about the aim and the risk of the study and signed a witnessed consent form. All volunteers were interned for 2 weeks before the beginning and during the whole time of the study in order to achieve adaptation and synchronisation.

The volunteers were put on a normal standard diet including a drink of 150 ml of mineral water

every hour between 8 and 22 o'clock. Alcoholic drinks, xanthine-containing beverages, and coffee were interdicted as well as food intake apart from set meal times (7.30–8.00 h breakfast; 13.00–13.45 h lunch; 19.00–19.45 h dinner). Sports were ruled out for the whole duration of the study.

None of the volunteers reported any history of allergy. Details of the volunteers are given in Table 1.

Blood samples were drawn from a 45 cm long subclavian catheter which had been atraumatically placed into the brachial vein. The catheter was maintained for the period of investigation. Plasma samples were obtained by centrifugation at +4° and stored at −20 °C for duplicate examination. Plasma histamine assays were performed according to the method of Lorenz [13].

The study was performed on two consecutive days in a blind design placebo versus tritoqualin. It consisted of both the evaluation of control plasma histamine levels and plasma levels during the administration of 3×200 mg tritoqualin. On day 1, all volunteers were given placebo, on day 2 tritoqualin. The medication was administered at 8.00 h, 16.00 h, and 23.00 h (group I) or 24.00 h (group II) respectively combined with the above mentioned 150 ml of mineral water. Tritoqualine levels were analysed fluorometrically according to the established method [14]. Data were processed by paired *t*-Test analysis for statistical significance.

Results

Mean plasma histamine levels obtained after treatment with placebo oszillate between 0.01 ng/ml (specifity of method) and 0.31 ng/ml following the treatment with placebo and between 0.01 ng/ml and 0.14 ng/ml following treatment with tritoqualin (Table 2). The mean diurnal plasma histamine levels decrease from 0.13±0.1 ng/ml (placebo) to 0.06±0.05 ng/ml (tritoqualine) (Table 3).

The three acrophases are 5.4±1.8, 12.8±0.6 and 19.3±0.8 following treatment with placebo and 4.1±1.6, 13.1±0.8, and 19.3±0.4 following treatment with tritoqualine. The bathyphases are 10.4±1.0 and 10.3±1.1, 16.5±1.9, and 15.8±1.2, 23.2±1.1, and 21.8±1.9 following treatment with placebo and tritoqualin respectively.

Table 1
Characteristics of the volunteers.

Nr.	Group	Volunteer initial	Sex	Age	Weight	Allergic history
01	I	E.B.	f	27	51	–
02	I	S.B.	m	28	70	–
03	II	C.M.	f	24	60	–
04	I	T.D.	m	25	72	–
05	I	J.E.	f	23	65	–
06	II	W.H.	m	25	72	–
07	I	E.F.	m	24	75	–
08	II	S.S.	f	22	56	–
09	II	M.W.	m	31	63	–
10	I	E.H.	f	25	58	–
11	II	I.K.	f	26	57	–
12	II	P.K.	m	24	75	–

Table 2
Mean plasma histamine levels of 12 healthy volunteers at different times after Placebo (P) and Tritoqualin (T) 200 mg t.i.d.

	P	T
7.00	0.21±0.07	0.11±0.07
8.00	0.10±0.04	0.01±0.01
9.00	0.08±0.05	0.07±0.05
10.00	0.04±0.04	0.02±0.02
11.00	0.04±0.04	0.01±0.01
12.00	0.16±0.10	0.07±0.05
13.00	0.19±0.13	0.08±0.04
14.00	0.05±0.05	0.06±0.05
15.00	0.17±0.06	0.01±0.01
16.00	0.02±0.01	0.01±0.01
17.00	0.14±0.08	0.02±0.02
18.00	0.08±0.06	0.04±0.03
19.00	0.19±0.07	0.08±0.05
20.00	0.07±0.03	0.07±0.05
21.00	0.12±0.06	0.05±0.08
22.00	0.04±0.04	0.03±0.02
23.00	0.08±0.05	0.03±0.02
24.00	0.06±0.05	0.04±0.04
1.00	0.13±0.09	0.09±0.04
2.00	0.11±0.05	0.08±0.07
3.00	0.31±0.07	0.12±0.05
4.00	0.17±0.04	0.13±0.02
5.00	0.27±0.05	0.14±0.03
6.00	0.13±0.03	0.10±0.05
7.00	0.23±0.08	0.11±0.04
	$p<0.001$	

The area under the plasma histamine level versus time curve (AUC) is reduced under treatment with tritoqualin. The mean reduction of free plasma histamine is about 50% (Table 4) expressed as

$$\frac{AUC_{placebo}-AUC_{tritoqualin}}{AUC_{placebo}} \cdot 100.$$

Table 3
Mean diurnal plasma histamine levels in 12 healthy volunteers following administration of Placebo and 600 mg Tritoqualin.

	P	T
1	0.17±0.10	0.08±0.06
2	0.14±0.10	0.07±0.05
3	0.12±0.05	0.04±0.05
4	0.18±0.09	0.08±0.07
5	0.20±0.12	0.07±0.06
6	0.07±0.06	0.03±0.04
7	0.15±0.11	0.08±0.05
8	0.09±0.08	0.06±0.05
9	0.09±0.07	0.06±0.05
10	0.21±0.14	0.05±0.04
11	0.06±0.05	0.04±0.05
12	0.07±0.05	0.07±0.05
Mean value:	0.13±0.10	0.06±0.05

The bioavailability of tritoqualin was demonstrated in plasma. The mean values of tritoqualin levels show an increase from 0.161±0.065 µg/ml to 1.56±0.51 µg/ml.

Discussion

It could be demonstrated that plasma histamine levels are subject to biorhythmic variations [12] in normal volunteers. The pattern of the plasma histamine versus time shows three maxima and minima. Whilst two of these maxima may be related to food uptake or governed by central nervous mechanisms, the third one occurs in early morning hours (acrophase 5.42± 1.83) as already observed in asthmatic patients. This pattern of biorhythmic changes which is observed following treatment with placebo is not altered by administration of tritoqualin. The plasma histamine level versus time curve shows a similar pattern after administration of placebo and tritoqualin respectively, but in the latter case on a significantly reduced plasma histamine level (Figure 1). The acrophases and bathyphases are only slightly different from those obtained under placebo. Beside circadiane fluctuations plasma histamine levels are nearly constant [16] and the correlation between free plasma histamine and total blood histamine is poor as shown by a correlation coefficient of 0.67 [17]. It may therefore be concluded that changes in plasma histamine were related to the drug influence.

Table 4
Areas under the plasma histamine level versus time curve.

01	T	0.235	0.595	0.59	1.42
	P	0.835	1.34	1.37	3.545
	$\frac{P-T\times 100\,(\%)}{P}$	72	56	57	60
02	T	0.245	0.175	0.96	1.38
	P	0.845	0.56	1.75	3.155
	$\frac{P-T\times 100\,(\%)}{P}$	71	69	45	56
03	T	0.225	0.135	0.56	0.9
	P	0.635	0.72	0.9	2.255
	$\frac{P-T\times 100\,(\%)}{P}$	65	81	38	59
04	T	0.205	0.3	1.14	1.645
	P	1.425	1.015	1.42	3.86
	$\frac{P-T\times 100\,(\%)}{P}$	85	70	20	57
05	T	0.235	0.31	0.88	1.425
	P	0.995	1.11	2.14	4.245
	$\frac{P-T\times 100\,(\%)}{P}$	76	72	59	66
06	T	0.19	0.08	0.47	0.74
	P	0.325	0.36	0.59	1.275
	$\frac{P-T\times 100\,(\%)}{P}$	42	78	20	42
07	T	0.15	0.39	0.9	1.44
	P	1.195	0.395	1.42	3.01
	$\frac{P-T\times 100\,(\%)}{P}$	88	9	37	52
08	T	0.185	0.3	0.8	1.285
	P	0.35	0.385	0.79	1.525
	$\frac{P-T\times 100\,(\%)}{P}$	47	22	0	16
09	T	0.425	0.175	0.45	1.05
	P	0.445	0.38	0.93	1.755
	$\frac{P-T\times 100\,(\%)}{P}$	5	54	52	40
10	T	0.245	0.35	0.55	1.145
	P	0.46	1.345	1.95	3.955
	$\frac{P-T\times 100\,(\%)}{P}$	63	76	72	71
11	T	0.205	0.085	0.63	0.92
	P	0.11	0.165	0.63	0.905
	$\frac{P-T\times 100\,(\%)}{P}$	–	49	0	–
12	T	0.27	0.445	0.59	1.305
	P	0.27	0.27	0.91	1.45
	$\frac{P-T\times 100\,(\%)}{P}$	0	–	35	10
$\frac{1}{n}E_T$		0.235 (19%)	0.278 (23%)	0.71 (58%)	1.22
$\frac{1}{n}E_P$		0.674 (26%)	0.67 (26%)	1.23 (48%)	2.58
$\frac{1}{n}E_{\frac{P-T}{P}}$		65%	59%	42%	53%

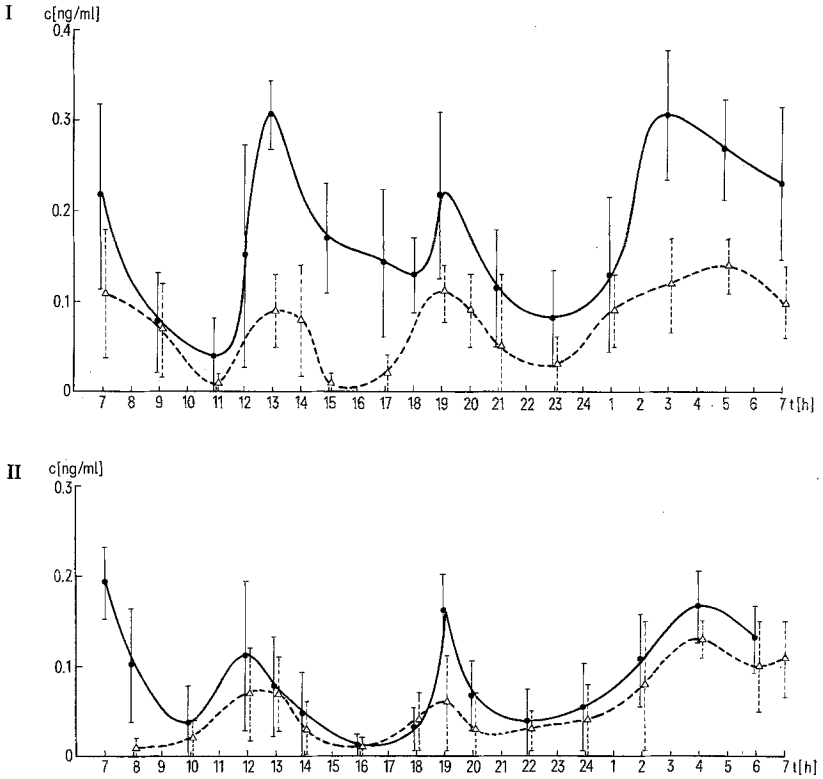

Figure 1
Plasma histamine levels after placebo (——) and tritoqualin (. . .) respectively. (Roman numbers represent groups I and II.)

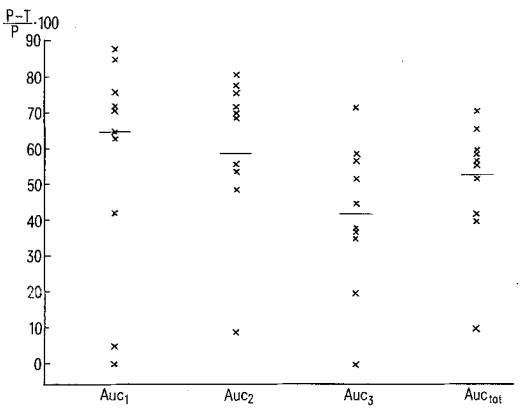

Figure 2
Reduction of plasma histamine levels after treatment with tritoqualin. AUC_n represent the areas under the curves between the bathyphases (see text).

It should be pointed out that the plasma histamine levels in all volunteers were reduced under the administration of tritoqualin. The reduction of plasma histamine levels shows statistical significance in 9 of 12 volunteers. Statistical significance could not be demonstrated for those volunteers with basically very low plasma histamine levels. Even though, the mean diurnal plasma histamine levels during treatment with tritoqualin turned out to be significantly lower than those during the placebo phase ($p < 0.001$). Both the minima and the maxima decreased during the tritoqualin phase.

The total amount of histamine in the plasma is represented by the area under the plasma histamine level versus time curve (AUC). The AUC_1 represents the area under this curve between the bathyphase 1 and 2, AUC_2 between bathyphases

2 and 3, AUC_3 between bathyphases 3 and 6 or 7 p.m. and AUC_{total} sums up the three differential AUC's. During treatment with placebo both AUC_1 and AUC_2 represent about 26% of the total plasma histamine, whereas AUC_3 totals about 48%. Following the administration with tritoqualin AUC_1 represents about 19%, AUC_2 about 23%, and AUC_3 about 58%. The reducing effect on the plasma histamine level by tritoqualin is about 65% on AUC_1, about 59% on AUC_2, and 42% on AUC_3. The total reduction of plasma histamine is about 53% (Figure 2).

Tritoqualin was thought to act as histamine decarboxylase inhibiting agent [5]. It was therefore surprising that it shows an acute, spontaneous effect on plasma histamine levels. Based on the HDC-concept this effect was expected to be sustained, because already synthesized and stored histamine could be excreted from the basophiles into plasma without drug interaction. Thus it might be concluded from our data that tritoqualin is not only/or not an HDC inhibiting agent, but other possible mechanisms of action must be taken into consideration. That meets the results of an experimental study regarding the inhibition of the specific acidic histamine decarboxylase from mice, rabbits, and men [15]. Based on our data we cannot decide whether tritoqualin induced catabolic processes on histamine or whether it inhibits its release from basophiles into plasma by stabilization of these histamine producing cells.

The findings of this study support existing knowledge about the efficacy of tritoqualin in the treatment of histamine dependent allergic diseases e.g. bronchial asthma, food allergy, and allergic rhinitis. Further experiments have to be done to elucidate the open mechanistic questions.

Received 14 November 1986; accepted 19 May 1987

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