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Immunological reactivity in ranitidine factory workers

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Abstract The involvement of immunological reactivity to ranitidine base (R-b) and ranitidine hydrochloride (R-HCl) in the development of occupationally related symptomatology was analyzed in 40 subjects employed in a pharmaceutical plant producing ranitidine and in 33 nonexposed controls, using a specific dose-response lymphocyte proliferative test (lymphocyte transformation test: LTT). Of the 40 workers, 11 (28%) gave positive reactions to LTT: 3/11 to R-b, 4/11 to R-HCl, and 4/11 to both compounds. None of the controls gave positive reactions. Cutaneous, oculonasal, or respiratory work-related symptoms were cited by 23 of the 40 (58%) subjects; ten of these 23 subjects (43%) were LTT positive. One asymptomatic case was LTT positive. The present results indicate that specific immune reactivity to ranitidine, analyzed by LTT, is associated with the presence of occupational symptomatology; R-HCl and R-b seem to share some antigenic determinants, because of the partial cross-reactivity shown by the examined compounds. Nonimmunological, probably irritative, mechanisms are also present in some of the symptomatic subjects.

Key words Ranitidine
N-N-Dimethyl-5-(2-(1-methyl-amino-2-nitrovinylamino) ethylthiomethyl) furfurylamine hydrochloride
H_2-receptor antagonist · Occupational sensitization
Lymphocyte transformation test

Introduction

Ranitidine, *N-N*-dimethyl-5-(2-(1-methylamino-2-nitrovinylamino)ethylthiomethyl)furfurylamine hydrochloride, is a furan derivative which acts as a competitive, reversible antagonist of histamine H_2 receptors; it is therapeutically employed as ranitidine hydrochloride (R-HCl) in the treatment of duodenal ulcers and gastric hypersecretory syndromes (Mills et al. 1991; Lancaster-Smith et al. 1991; Penston and Wormsley 1992). This drug is generally well tolerated; however, minor side-effects, such as headache, dizziness, and constipation (Eandi et al. 1990) and anaphylactic or anaphylactoid reactions including rashes, urticaria, laryngeal spasm, and respiratory symptoms (Brayko 1984; Greer and Fellows 1990; Picardo and Santucci 1983; Simon et al. 1982; Lazaro et al. 1993) have been reported. Haboubi and Asquith (1988) studied three cases of vasculitis with vascular deposits of IgA and C3 in ranitidine-treated patients. Various authors (Rycroft 1983; Goh and Ng 1984; Alomar et al. 1987; Romaguera et al. 1988, 1990) have also described work-related cutaneous or respiratory symptoms suggestive of IgE-mediated or delayed hypersensitivity-type immunological reactions.

In this study we evaluated a series of 40 workers exposed to ranitidine processing in a chemical plant, in order to determine whether specific sensitization, detected in vitro, could be associated with work-related symptomatology. For preliminary screening we employed the lymphocyte transformation test (LTT), a nonsensitizing test able to reveal specific immune recognition and reactivity to the drug, although not discriminating among the different types of immune reactions.

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Materials and methods

The process

The manufacturing process in the examined factory starts from ranitidine base (R-b) to produce various therapeutic commercial preparations, through two separate steps carried out in different areas. In the first stage, bulk quantities of R-b are treated with HCl to form the saline compound R-HCl, which is subsequently granulated. In the second stage, R-HCl granules are sieved, assembled in preparations for clinical use (tablets or vials), and packed. The

procedures are carried out in nearly closed systems, except for two steps, the pouring of R-b into reaction tanks for HCl treatment (first stage) and the sieving phase (second stage). Although workers constantly wear protective suites, accidental inhalation or contamination of unprotected skin is possible.

The mean air dustiness in the work rooms (conversion-granulation phase) was 5 $\mu g/m^3$, and dust dumpers were employed during nonclosed manufacturing processes.

Subjects

In this study we investigated a series of 40 workers, 37 males and three females, engaged in the ranitidine processing. Nineteen subjects working in the conversion-granulation stage had contact with both R-b and R-HCl, while 21 employed in the sieving-packing stage had contact only with R-HCl. All subjects were regularly rotated among the various steps in the processing line for the manufacturing stage in question. At the time of the study 33 persons were currently exposed to ranitidine, while seven were no longer exposed (the interval between the last exposure and blood collection ranged between 4 months and 5 years). Exposure time ranged from 7 months to 9 years (mean 4.7 years) for currently exposed subjects and from 7 months to 5 years for workers no longer exposed (mean 2.4 years). The medical evaluation included an initial interview by a physician and a standardized questionnaire designed to obtain information on medical history and work-related reactions, with special regard to cutaneous, conjunctival, nasal, and bronchial symptoms and to hypersensitivity reactions. The occurrence of accidental heavy exposure and the therapeutic use of ranitidine were also checked.

A control group of 33 healthy blood donors was also examined; it was ascertained that none of these subjects had received previous ranitidine treatment.

Cell culture technique and the LTT

Venous heparinized blood was drawn aseptically from patients and controls. Lymphocytes were isolated on a Ficoll gradient (Ficoll-Hypaque, Pharmacia Fine Chemicals), washed, and resuspended at 1×10^6/ml in RPMI 1640 medium (Bio-Chrom) supplemented with L-glutamine 10 nM and 10% fetal calf serum (Bio-Chrom). Quintuplicate samples were cultured in flat-bottomed microtiter plates containing 2×10^5 lymphocytes per well in 200 µl of culture medium. R-b or R-HCl was added at a final concentration of 0.05 µg/ml, 0.1 µg/ml, 1.0 µg/ml, 10 µg/ml, or 100 µg/ml, and lymphocytes were incubated in 5% CO_2 at 37°C for 72 and 120 h.

As a functional control, cells were stimulated by the polyclonal mitogen phytohemagglutinin (PHA, Wellcome), at an optimal concentration (10 µg/ml). After the addition of 0.5 µCi of ^{3}H-thymidine (Radiochemical Centre, Amersham) to each well, the cells were incubated for an additional 6 h and harvested; ^{3}H-thymidine incorporation was determined by liquid scintillation. Results were expressed as a stimulation index (SI), from the following formula:

cpm with ranitidine/cpm without ranitidine = SI.

Stimulation was considered positive if the SI was $\geqslant 2.0$ at any point in a dose-response curve, at 72 and/or 120 h of incubation.

Results

Clinical evaluation

A personal history of atopy was present in 5/40 (13%) workers. No former skin or respiratory disease was ascertained. Four (10%) subjects had previously been treated with ranitidine. When reactions due to ranitidine exposure were evaluated, 23/40 (58%) workers reported the symptoms shown in Table 1. Skin and nasal mucosa were mainly involved, although severe reactions comprising dyspnea and laryngeal edema were present in two cases. Symptoms worsened on weekdays and lessened at weekends or during vacations. When the job was related to symptomatology, it appeared that 10/19 (53%) persons working in the first-stage department and 13/21 (62%) working in the second-stage department developed symptoms. Moreover, three symptomatic subjects reported a personal history of atopy and two of four subjects therapeutically treated with ranitidine became symptomatic when professionally exposed.

Lymphocyte transformation test

A normal response to lymphocyte stimulation with PHA was elicited in all the subjects tested (exposed workers and controls). When LTT was performed with ranitidine, 11/40 (28%) workers gave a positive response and 2/40 (5%) were borderline. Lymphocytes from control subjects did not react with ranitidine. Table 2 reports lymphocyte reactivity to either R-b or R-HCl: seven subjects were LTT-positive to R-b, eight to R-HCl, and four of them recognized both compounds; borderline individuals reacted only with R-b.

Culture time strongly influenced LTT results, the best reactivity being apparent at the longer incubation time (120 h). Thus eight subjects were positive after 120 h, two at both 72 h and 120 h, and only one after 72 h. Table 3 shows the relationship between LTT reactivity, expressed as SI, and ranitidine (R-b and R-HCl) concentration at 120-h incubation. The two lowest concentrations (0.05 µg/ml and 0.1 µg/ml) failed to elicit a significant response, except in subject T. G. Optimal SIs were achieved at 10 µg/ml. Ten out of 11

Table 1 Symptomatology of ranitidine-exposed workers

Symptoms	No. of subjects
Cutaneous (20 subjects)	
Local or generalized pruritus	17
Erythema	6
Eczema	3
Crusting of nasal mucosa	3
Urticaria	1
Oculonasal (14 subjects)	
Sneezing, rhinorrhea, nasal congestion	11
Red and/or itching eyes	3
Respiratory (3 subjects)	
Cough	2
Laryngeal edema	1
Dyspnea	1

Table 2 Lymphocyte reactivity to ranitidine base (R-b) and ranitidine hydrochloride (R-HCl) in exposed subjects, expressed as net cpm (cpm with ranitidine − cpm without ranitidine). The highest value at 120-h incubation is reported. [*SI* stimulation index (cpm with ranitidine/cpm without ranitidine)]

Worker	Response to LTT (net cpm)	
	R-b	R-HCl
T.G.[a]	4960 (SI 4.1)	5678 (SI 4.6)
C.G.[a]	Neg.	5148 (SI 3.3)
L.R.	Neg.	2450 (SI 2.6)
B.J.	1321 (SI 2.5)	Neg.
R.L.	1971 (SI 2.3)	Neg.
P.G.	2708 (SI 2.3)	2478 (SI 2.2)
T.A.	1893 (SI 2.3)	1612 (SI 2.1)
O.R.	Neg.	896 (SI 2.3)
T.P.[b]	1221 (SI 2.2)	Neg.
F.G.	Neg.	971 (SI 2.2)
G.G.	1570 (SI 2.1)	1602 (SI 2.2)
B.L.	3378[c] (SI 2.0)	Neg.
F.I.	2368[c] (SI 2.0)	Neg.

[a] Positive at both 72-h and 120-h incubation
[b] Culture time = 72 h
[c] Borderline values

Table 3 Positivity to LTT at various R-b and/or R-HCl (italicized) concentrations at 120-h incubation. Results are expressed as stimulation index (SI = cpm with ranitidine/cpm without ranitidine)

Subject	Ranitidine concentration (μg/ml)				
	0.05	0.1	1.0	10	100
B.J.	1.1	1.2	1.3	2.5	2.1
	1.8	*1.9*	*1.5*	*1.6*	*1.2*
R.L.	1.4	1.5	2.0	2.3	1.6
	0.9	*0.9*	*0.8*	*1.1*	*1.0*
T.P.	1.1[a]	1.5	2.2	2.0	2.0
	1.2[a]	*1.0*	*1.2*	*1.3*	*1.2*
C.G.	1.1	1.2	1.3	1.6	1.5
	1.3[b]	*1.6*	*1.9*	*3.3*	*2.9*
F.G.	1.0	1.0	1.4	1.8	1.4
	0.7	*0.8*	*1.7*	*2.2*	*1.9*
L.R.	1.1	1.2	0.9	1.5	1.9
	1.5	*1.6*	*1.5*	*2.3*	*2.6*
O.R.	1.0	1.1	1.0	1.1	1.8
	0.6	*0.6*	*0.8*	*1.3*	*2.3*
G.G.	1.4	1.4	1.8	2.1	2.0
	0.8	*0.9*	*1.2*	*2.2*	*2.0*
P.G.	0.9	0.9	1.3	2.3	1.9
	1.0	*1.1*	*1.9*	*2.2*	*1.7*
T.G.	2.0[b]	1.9	3.9	4.1	4.1
	1.5[b]	*2.5*	*2.9*	*4.6*	*3.0*
T.A.	0.9	1.2	1.5	2.2	2.3
	1.2	*1.3*	*1.6*	*2.0*	*2.1*

[a] Culture time = 72 h
[b] Positive at both 72-h and 120-h incubation

(91%) LTT-positive subjects had work-related symptoms; six of them worked in the first-stage department and four in the second-stage department. Table 4 summarizes the exposure-related and clinical data of subjects with positive or borderline reactions to the ranitidine compounds. Seven subjects were exposed to ranitidine at the time of testing, while four symptomatic ones, complaining of work-related symptomatology, were no longer exposed. All LTT-positive workers were exposed to R-HCl, and seven were also exposed to R-b. Eight subjects reacted with R-HCl and seven with R-b. Various reactivity patterns were observed: three of seven subjects exposed to both R-HCl and R-b recognized both compounds, while four reacted with only one molecule (two with R-HCl and two with R-b); two of four persons exposed only to R-HCl reacted in vitro with this compound, while one reacted with both molecules and one with R-b. Borderline subjects were exposed to both compounds and showed a weak reaction to R-b. Work-related symptoms were present in ten LTT-positive cases; the duration of the exposure period did not appear to correlate with the development of symptomatology and sensitization, while the severity of symptoms did not correlate with the kind of exposure (one or both ranitidine compounds) or LTT response.

Table 5 reports the exposure-free period, responses to LTT, and kind of symptomatology in seven workers who ceased exposure because of occupational reactions. It can be noted that the length of the exposure-free period did not affect the LTT results, as four of seven individuals were positive and three of them reacted with both compounds; moreover, the highest LTT reactivity of the series was observed in one of these subjects, T. G. (SI shown in Table 3).

Discussion

In this study we employed LTT, a reliable in vitro assay indicative of immune recognition and reactivity, although not immunopathological type-specific, with the aim of assessing specific sensitization to ranitidine in a series of 40 occupationally exposed persons.

The determination of immune reactivity to ranitidine is usually performed by in vivo techniques, such as prick or patch tests. Picardo and Santucci (1983) postulated an IgE-mediated mechanism in a patient reactive to oral administration of ranitidine and positive when prick-tested with the drug. Lazaro et al. (1993) reported a case of anaphylactic reaction to therapeutic ranitidine that was positive to prick and intradermal skin tests but negative to in vitro analysis for IgE-mediated reactions.

Patch tests, however, have been performed by most authors, such as Alomar et al. (1987), Goh and Ng (1984), and Romaguera et al. (1988, 1990), who described an interesting case positive not only to occupational compounds but also to two intermediate

Table 4 Exposure-related and clinical data of LTT-reactive subjects

Subject	Exposed to		Exposure period (years)	LTT response		Symptomatology
	R-b	R-HCl		R-b	R-HCl	
B.J.	+	+	7	+	−	Pruritus
R.L.	−	+	8	+	−	Pruritus Sneezing Rhinorrhea
T.P.	+	+	7	+	−	Negative
C.G.	−	+	9	−	+	Pruritus Sneezing Rhinorrhea Nasal congestion
F.G.	−	+	4	−	+	Rhinorrhea
L.R.	+	+	5[a]	−	+	Pruritus Erythema Cough, dyspnea Red eyes
O.R.	+	+	5	−	+	Pruritus Sneezing Rhinorrhea
G.G.	+	+	5[a]	+	+	Pruritus Erythema
P.G.[b]	+	+	2	+	+	Sneezing Rhinorrhea Cough, red eyes
T.G.	+	+	2[a]	+	+	Pruritus Erythema, eczema
T.A.	−	+	0.6[a]	+	+	Pruritus Erythema, eczema Laryngeal edema Red eyes
B.L.[c]	+	+	3	+/−	−	Negative
F.I.[c]	+	+	7	+/−	−	Negative

[a] No longer exposed
[b] Orally treated with ranitidine
[c] Borderline cases

Table 5 Exposure-free period, LTT reactivity, and symptomatology in seven previously exposed workers

Subject	Exposure-free period (years)	LTT reactivity		Symptomatology
		R-b	R-HCl	
B.G.	0.3	−	−	Pruritus Eczema
F.M.	3	−	−	Pruritus Eczema
S.R.	1	−	−	Pruritus Sneezing Nasal congestion
L.R.	2	−	+	Pruritus Erythema Cough, dyspnea Red eyes
G.G.	2	+	+	Pruritus Erythema
T.G.	5	+	+	Pruritus Erythema Eczema
T.A.	5	+	+	Pruritus Erythema, eczema Laryngeal edema Red eyes

molecules. All these reports describe single cases (Picardo and Santucci 1983; Alomar et al. 1987; Goh and Ng 1984; Romaguera et al. 1990; Lazaro et al. 1993) or small series of symptomatic individuals (Romaguera et al. 1988); to our knowledge, this is the first study showing the occurrence of specific immune responses in a larger population of exposed individuals, both symptomatic and asymptomatic. Positive LTT reactions were found in 11 subjects, and ten of these had occupational symptoms; high LTT reactivity, however, was not associated with severe symptomatology. Only one asymptomatic case was LTT-positive and may need a clinical follow-up if exposure is not discontinued. Previous therapeutic administration did not appear a main sensitizing agent, as only one of four treated individuals developed occupational reactions along with a positive LTT. The presence of negative symptomatic subjects suggests a nonimmunological, possibly irritative pathogenesis.

The type of symptomatology did not correlate with the kind of duties or with the presence of an immune response; rotation of workers among the various stages of the production process may partly explain this fact,

while phenomena such as pruritus, erythema, rhinitis, eye redness, and inflammation of the respiratory mucosa may be ascribed to either immune or irritative reactions. In our series, immune system involvement was revealed in ten out of 23 symptomatic subjects, showing a relatively high frequency of sensitizing capacity. The underlying immunopathological mechanism was beyond the scope of the present study. It is noteworthy that even when the symptoms were clinically suggestive of IgE-mediated mechanisms, no correlation was found with a personal history of atopy, in accordance with other authors' findings (Picardo and Santucci 1983; Rycroft 1983; Goh and Ng 1984). Eczematous lesions, present in two individuals, may be attributable to delayed hypersensitivity phenomena.

Previous reports (Picardo and Santucci 1983; Alomar et al. 1987; Goh and Ng 1984) suggest that the furan ring, present either in intermediate or final products, may be the main sensitizing group of the ranitidine molecule. On the other hand, Lazaro et al. (1993) describe a case positive to skin test with ranitidine, but not cross-reacting with nitrofurantoin containing the furan group. Moreover, Rycroft (1983) refers to a chemist sensitized to the intermediate diamino product in the synthesis of ranitidine but unresponsive to R-HCl, and points to the terminal unsubstituted amino group of this compound as the relevant sensitizing agent. Examination of the response patterns of our subjects suggests the antigenic determinants to be partially different in the two forms of ranitidine examined; further studies are needed to discriminate the shared from the unshared specificities. Industrial ranitidine production employs almost completely closed systems and protective measures are provided when direct contact is possible; it is therefore difficult to establish how workers may become sensitized. It may be postulated that accidental contamination occurs from contact with momentarily unprotected skin or from penetration of protective devices by the allergen; in fact, most cases of sensitization in industry (Goh and Ng 1984; Romaguera et al. 1988) occur in workers involved in the stage of the process where bulk quantities of R-b are openly handled with the aid of protective clothing; however, low-dose exposure to ranitidine is also likely to induce sensitization, as reported by Rycroft (1983), Alomar et al. (1987), and Romaguera et al. (1990) and as confirmed in our series, where three out of three chemical analysts became sensitized and developed immunological lesions.

In conclusion, in this study we found that 23 out of 40 individuals professionally exposed to ranitidine reported an occupational symptomatology; immune reactivity, analyzed by LTT, was strictly related to the presence of symptoms. It must be noted that whereas LTT proves sensitization to the drug, it does not indicate the presence of a clinical reaction or the underlying immunopathological mechanism; it does indicate, however, that positive subjects are at risk of reactions upon reexposure to the specific agent, and LTT therefore may be considered a safe in vitro test to assess the need for avoidance of reexposure in the working environment.

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