

Leucocyte Migration Test and Hypersensitivity to Glafenin

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The leucocyte migration test (LMT) was performed on 20 patients with an intolerance to glafenin – a non-narcotic analgesic drug. LMT was found to be positive in 50% of the subjects with intolerance, a highly significant percentage as compared with the control groups. HSA-glafenin was found to be the most appropriate method for presenting the antigen, but glafenin and its hydroxylated metabolites were only found to induce a migration inhibition in the subjects intolerant to glafenin.

Key words: drug hypersensitivity; glafenin; leucocyte migration test.

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Glafenin (Fig. 1) is a highly active non-narcotic analgesic agent. Although this drug is now widely used in several European countries, side effects are rare (10). These are mainly of the "allergic type", i.e. urticaria or a syndrome mimicking anaphylactic shock (2,5,8,10) (urticaria, malaise, hypotension). The pathogenesis of these side effects, most often occurring as soon as 20 min after an

oral intake of the drug, has so far not been established. It was thus necessary to adapt *in vitro* tests for the study of patients with a glafenin intolerance. The LMT has been found to be a useful method for the *in vitro* study of drug hypersensitivity, especially penicillin (12,16,17), even in subjects with type I hypersensitivity (11).

In this paper, we report on a study in which

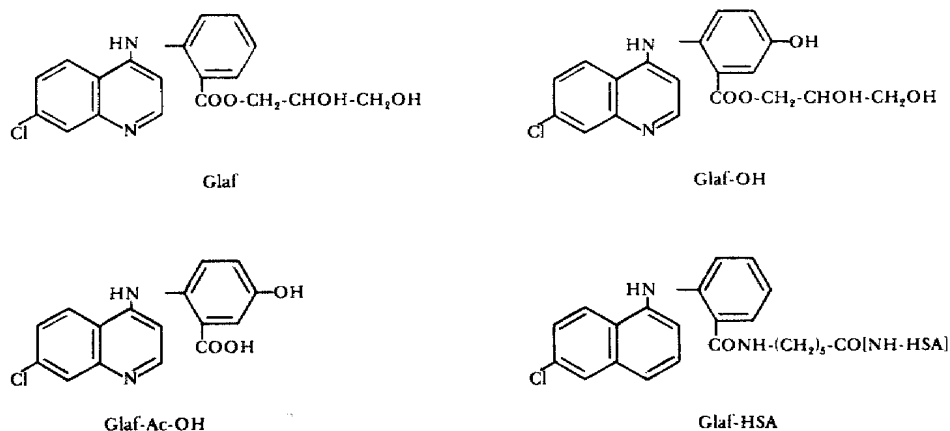


Figure 1. The four types of glafenin antigens.

the LMT has been used with several presentations of glafenin in patients with and without an intolerance to this drug.

MATERIAL AND METHODS

Patients. Sixty subjects were tested:

(i) Twenty patients (5 males, 15 females, average age 33 years, ranging from 20 to 72) with a well-documented intolerance to glafenin: six urticaria and hypotension ("anaphylactic shock"); seven urticarias; three pruritus and malaise; four maculopapular rashes. Glafenin was considered as the causative agent because it was the only drug absorbed in all cases; moreover, in five cases a subsequent ingestion of glafenin reproduced the symptoms.

(ii) Twenty-five patients (16 males, 9 females, average age 39, ranging from 25 to 67) without intolerance to glafenin; none had taken this drug for 1 year before the blood was taken.

(iii) Fifteen patients without intolerance to glafenin were tested 5 to 8 days after a well-tolerated treatment (8 days; 600 mg/day/per os).

Leucocyte migration test. The technique used has been described previously; it was similar to Federlin's (6) which is a slight modification of the method described by Søborg & Bendixen (18). The criteria for a positive LMT have been reported previously (15). Briefly, a 0.80 migration index (i.e. migrations with antigen/migration without antigens) was considered to be the highest value for a positive test. The reasons for choosing this value have been discussed previously (15, 16). The significance of each inhibition was further assessed as follows: for each control migration series, the mean (MC) and the standard deviation (s.d.) were calculated; in each experiment reported as positive, the mean migration value with antigens (MA) was $MA \leq MC - 3 \text{ s.d.}$ (i.e. significant at the 0.01 level). In a few cases, a stimulation of the migration was observed; it was only noted as such and outlined when $MA > MC + 3 \text{ s.d.}$

Antigens. Four types of glafenin antigens were used in this study: (Fig. 1) Glafenin alone (Gla), hydroxylated compounds such as, Gla-OH (RU 23709) and Gla-OH (RU 24758) and a protein-linked compound, Gla-human serum albumin (Gla-HSA = RU 23710). The two hydroxylated compounds, Gla-OH and Gla-OH, were prepared by the classical procedures (1). The compound Gla-HSA was obtained by using 6-2-(7-chloro 4-quinolinylamino) benzoyl amino hexanoic acid, according to the mixed

Table 1 Leucocyte migration test (migration indexes) with glafenin antigens in untreated subjects

	Gla-HSA	Gla	Gla-OH	Gla-OH
	1.06			
	0.98			
	1.01			
	0.90			
	0.93			
	0.91			
	0.82			
	0.93	1.13		
	0.88	0.95		
	1.00	1.10		
	0.86	0.88		
	0.97	0.89		
	0.89	0.89		
	0.83	1.17		
	0.91	1.15		
		1.00	0.94	0.93
	1.11	1.03	0.96	1.03
	0.83	1.03	0.96	0.90
	0.91	0.90	0.90	0.98
	1.04	1.08	0.96	0.97
	0.91	1.05	0.88	0.96
	0.91	1.04	0.90	0.95
	0.96	1.15	0.94	1.06
	0.96	0.92	1.05	1.12
	0.88	1.01	1.00	1.05
Total	24	18	10	10
Positive Nb	0	0	0	0
%	0	0	0	0
Sensitized subjects %			0	

Gla-HSA, Gla, Gla-OH, Gla-OH see Fig. 1.

Table 2. Leucocyte migration test (migration indexes) with glafenin antigens in treated subjects

	Glaf-HSA	Glaf	Glaf-OH	Glaf-Ac-OH
	<i>0.77</i>	1.01		
	<i>0.61</i>	0.88	0.88	0.93
	0.87			
	1.05			
	0.82	1.03		
	0.93	0.92		
	0.97	1.15		
	1.12	1.01	0.84	0.98
	1.03	1.07		
	0.99	0.88	0.95	0.98
	1.16	0.88		
	1.04	1.00	1.05	1.04
	0.96	0.94	1.12	0.89
	0.90	1.13	1.02	1.02
	0.97	0.94	0.94	0.95
Total	15	13	7	7
Positive Nb.	2	0	0	0
%	13	0	0	0
Sensitized subjects %		13		

Italicized numbers = significant migration inhibition.

anhydride technique of Erlanger et al. (4). The acid was prepared by treating ethyl γ -amino caproate with the chloride of 2-(7-chloro 4-quinolinylamino) benzoic acid and saponifying the resulting ester. Glaf-HSA thus contains 14.5 linked groups per HSA molecule.

Antigens were used in TC 199 + 10% horse serum. Preliminary studies have shown that the optimal concentration of Glaf-HSA was 50 mcg/ml. This dose was then used as a routine in this study; control migrations were performed with 50 mcg/ml of HSA which had been prepared as for Glaf-HSA but without the addition of glafenin.

The three other antigens (Glaf, Glaf-OH, Glaf-Ac-OH) were used at a concentration of 5 mcg/ml which corresponds to the number of Glaf groups present on 50 mcg/ml of Glaf-HSA.

Table 3. Leucocyte migration test (migration index) with glafenin antigens in patients with intolerance

Clinical symptom	Glaf-HSA	Glaf	Glaf-OH	Glaf-Ac-OH
I	<i>0.77</i>	1.17	1.02	0.97
Pseudo anaphylactic shock	0.90	1.04	0.98	1.00
	1.09	0.96		
	<i>0.70</i>	0.89	0.83	1.01
	1.02	<i>0.75</i>		
	0.92	<i>0.79</i>		
II	0.87	1.04	1.04	1.06
Urticaria	<i>0.79</i>	<i>1.23</i>	1.15	<i>1.20</i>
	0.84	<i>0.76</i>	1.03	0.94
	1.04	0.90	0.83	0.83
	<i>0.74</i>	1.02	<i>0.62</i>	<i>0.80</i>
	<i>0.59</i>	<i>0.74</i>	0.82	<i>0.75</i>
	0.93	0.96	1.01	0.95
III	0.97	1.08	1.07	1.12
Pruritus and malaise	0.90	<i>0.40</i>	0.90	1.00
	0.90	0.97		
IV	0.98	1.10	0.96	0.96
Maculo-papular rash	0.92	1.04		
	<i>0.68</i>	0.97	0.99	0.96
	0.80	0.99		
Total	20	20	14	14
Positive Nb.	6	6	1	3
%	30	30	7	21
Sensitized subjects %			50	

Italicized numbers = significant migration inhibition.

RESULTS

Table 1 shows that there was no significant inhibition of cell migration in the non-treated control group. In the treated control group (Table 2) there were only two positive LMT, both with Glaf-HSA; the other antigen presentations did not induce any significant inhibition.

The group of patients with an intolerance to glafenin was different in two respects:

(i) A positive LMT was found in 10 out of 20 cases (50%), a finding which is highly significant as compared with the non-treated controls ($P < 0.001$; χ^2), and even with the treated controls ($0.02 < P < 0.05$; χ^2).

(ii) Inhibition of cell migration was obtained not only with Glaf-HSA (six cases) but also with glafenin (six cases) and the hydroxylated metabolites (three cases and one case). This is a significant difference as compared with the two control groups ($0.02 < P < 0.05$; χ^2).

There was no correlation between a positive LMT with an antigen presentation and the clinical type of intolerance (Table 3).

DISCUSSION

This study clearly shows that it has been possible to distinguish *in vitro*, by means of the LMT, a group of patients intolerant to glafenin and two groups of patients without intolerance to this compound.

The rate of positive LMT in patients without intolerance was very low compared with other studies dealing with penicillin (17). Penicillin is a highly "allergenic" drug and the rate of positive LMT in subjects without clinical hypersensitivity to this drug was as high as 50% after a recent treatment (12,17). After a glafenin treatment only two out of 15 patients were found to have a positive LMT. This can reflect the fact that glafenin is a poor allergenic drug and could explain the rarity of allergic side effects after its use. Indeed, we have found that the less a drug was known to induce allergic reactions, the less the LMT was positive after a treatment in non-allergic subjects (13).

In patients with glafenin intolerance, there was a high rate of positive LMT. Moreover, antigenic presentations, which never induced a positive LMT in the control groups, were found to inhibit leucocyte migration in patients with intolerance to glafenin. This fact may reflect a different kind of immunization in patients with intolerance, but needs further study.

The LMT is able to distinguish patients with clinical symptoms of hypersensitivity, which are likely to belong to the "type 1" in the classification of Gell & Coombs (7). It may be that sensitization detected by means of the

LMT has nothing to do with the occurrence of the clinical symptoms. However, the highly significant difference between the three groups of patients contradicts this interpretation. LMT is thought to measure the release of the leucocyte inhibitory factor (LIF) by sensitized lymphocytes (3,14,19). The role of LIF *in vivo* has not been well established. Ortiz-Ortiz et al. (11) have previously found that the LMT was positive in patients with immediate type hypersensitivity to penicillin. However, we have to await further evaluation of the role of LIF in hypersensitive conditions before we can extrapolate for the clinical relevance of our findings.

The evaluation of other *in vitro* systems more closely correlated with immediate hypersensitivity, such as histamine release by circulating basophils, must also be made in patients with hypersensitivity to glafenin (9).

Nevertheless, we have demonstrated that the LMT is a useful technique for the study of these patients and we have evaluated the type of antigenic presentation to be used *in vitro*.

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