CHLORPROPAMIDE-ALCOHOL FLUSHING IN NON INSULIN-DEPENDENT DIABETES: PREVALENCE OF SMALL AND LARGE VESSEL DISEASE AND OF RISK FACTORS FOR ANGIOPATHY

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Chlorpropamide-alcohol flushing (CPAF) has been reported to be associated with a low prevalence of large and small vessel disease in non insulin-dependent diabetics^{3, 19, 20}. However, the specificity of the flushing test has recently been questioned^{17, 22}, and the occurrence of the CPAF phenomenon varies considerably in different diabetic clinics^{3, 12, 17, 19, 20, 22}.

We have studied the CPAF phenomenon in 108 non insulin-dependent diabetics and have investigated several so-called 'risk factors' for vessel disease (blood pressure, serum lipids and hemostatic parameters), to ascertain whether an uneven distribution of lesions might be related to metabolic or hemostatic abnormalities.

PATIENTS AND METHODS

One hundred and eight non insulin-dependent diabetics diagnosed according to the criteria of the National Diabetes Data Group²³ (56 females and 52 males, mean age 56 years, range 32-81) were asked to take a 250-mg chlorpropamide tablet in the evening, 12 h before the test and fast until the following morning, when they came to the clinic and drank 40 ml of sherry (Tio Pepe). Facial temperature was measured immediately before sherry ad-

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ministration and 20 min later, using two small sensor probes fixed by adhesive tape over the forehead and the lateral canthus of the eye, and a digital thermometer for registration. The test was positive if facial flush appeared and there was a sensation of warmth of the face and neck. The mean temperature increases in positive patients were $1.4^{\circ}\text{C} \pm 0.85$ (SD) and $0.4^{\circ}\text{C} \pm 0.15$ (SD) in negative patients. The room temperature was constant at 17°C (experiments were performed in a fully conditioned area), and the patients were kept waiting 1 h before drinking the sherry.

All the positive patients repeated the alcohol test three months later, with placebo given instead of chlorpropamide.

Clinical findings - The eyes of the 108 patients were examined independently through dilated pupils by two expert ophthalmologists unaware of their status as flushers. Patients with no retinal lesions or with fewer than 5 exudates or hemorrhages are pooled together in the nil/minimal group; patients with proliferative retinopathy are not considered separately, as there are too few of them²². Patients with severe retinopathy (more than 5 lesions in at least one eye) are subdivided on the basis of presence or absence of important visual loss (more than 50%, objectively measured) due to diabetic maculopathy.

Standard electrocardiography was performed: the presence of more than 3 extrasystoles over a 2-min registration, the presence of atrial fibrillation, the presence of left or right bundle branch block, the presence of an atrio-ventricular conduction time equal or greater than 0.21 sec, were considered pathological findings and pooled together in the 'rhythm or conduction defects'; patients with classical findings of previous myocardial infarction¹³ or showing a J-point depression of the S-T segment greater than 1.0 mm or a J-point elevation greater than 1 mm were considered ischemic; the lowering or flattening of the T wave with normal S-T segment were not considered as myocardial ischemia whereas the T-wave inversion even if not accompanied by S-T segment alterations was considered a sign of ischemia.

Oscillography was also performed in our subjects at both calves. The findings were classified as abnormal according to the following criteria. A maximal wave amplitude smaller than 5 mm, a maximal wave amplitude reduction in one leg compared to the other of more than 50% were considered to be reduced pulses, whereas the absence of wave was classified as abolished pulse.

Blood pressure was measured at the time of the first alcohol challenge, after 3 min in the supine position: systolic blood pressure was taken at the first Korotkoff's sound and diastolic blood pressure at the fourth Korotkoff's sound.

Blood sampling - One week after the first alcohol test, the patients came back to the clinic after an overnight fast; a blood sample was drawn through a 19 gauge needle for lipid and hemostasis assays.

Lipid determinations - Serum cholesterol was measured by the enzymatic method of BABSON et al.², serum triglycerides by the method of EGGSTEIN¹¹, serum HDL-cholesterol with phosphotungstate precipitation method of BURNSTEIN et al.⁵.

Hemostatic variables - Factor VIII coagulant activity (VIII:C) was assayed in citrated plasma by a two-stage method⁹ and factor VIII-related antigen (VIII R:Ag) by quantitative immunoelectrophoresis¹⁸ using a monospecific antiserum to human Factor VIII/von Willebrandt Factor from rabbit (Istituto Behring, Scoppito/L'Aquila, Italy). The results were expressed as percentage of plasma pooled from 30 normal subjects, snapped frozen and stored at -70 °C.

Plasma fibrinogen was determined by the method of VERMYLEN et al.²⁶ and values are expressed in g/dl. For plasma β -thromboglobulin (β -TG) measurement 2.7 ml of venous blood were immediately transferred into plastic tubes placed on crushed ice and mixed with 0.3 ml of an antiplatelet anticoagulant freshly prepared by adding 10 μ g of prostaglandin E₁ and 50 mg of theophylline to 10 ml of 10% EDTA⁴. β -TG was measured in platelet-poor plasma by radioimmunoassay using a commercial kit (Radiochemical Center, Amersham, U.K.), and the values expressed as ng/ml.

Glycosylated hemoglobin (HbA1) was measured using a commercial kit (Fast Hemoglobin Test System, Isolab Inc., Akron/O. U.S.A.) which pools together fractions HbA1 (a+b+c).

Statistical analysis was done by the χ^2 -test and the standard Student's *t*-test for unpaired data.

RESULTS

Table 1 shows the CPAF-positive and negative patients (numbers and percentages). Patients who had positive alcohol test after placebo administration, are considered aspecific flushers (AF), whereas those who had positive

	CPAF-positive (true flushers)	CPAF-negative (non flushers)	AF (aspecific flushers)	total flushers (CPAF-positive plus AF)
total number (%)	19 (17%)	73 (68%)	16 (15%)	35 (32%)
men	4 (21%)	43 (59%)	5 (31%)	9 (26%)
women	15 (79%)*	30 (41%)	11 (69%)	26 (74%)*
type of treatment				
- diet alone	10 (53%)	26 (60%)	1 (6%)	11 (31%)
- oral agents	9 (47%)	47 (40%)	15 (94%)	24 (69%)
presence of family history of diabetes	9 (47%)	30 (41%)	8 (50%)	17 (49%)
duration of diabetes (years, mean $\pm$ SD)				
- total	5.0 $\pm$ 4.1	7.4 $\pm$ 6.1	9.2 $\pm$ 5.4	6.5 $\pm$ 5.9
- women	4.92	7.4	9.6	6.9

* the percentage of women is significantly increased in flushers (both AF and CPAF-positive) ($p < 0.02$)

Tab. 1 - General characteristics of the 108 non insulin-dependent diabetics, divided according to their flushing status.

alcohol test after chlorpropamide administration and negative test after placebo, are indicated as CPAF-positive patients.

Of 35 flushers in the first test, 16 (46%) were aspecific and 19 (54%) were 'true' flushers. The percentage of women is significantly higher in the CPAF-positive and in the AF group ($p < 0.02$), suggesting that flushing phenomena may be sex-linked. The presence of a family history of diabetes was equally distributed in all the groups.

The mean duration of diabetes did not significantly differ among groups, even though it was slightly shorter for the 'true' flushers and slightly longer for the AF than for the CPAF-negative patients. Table 1 also shows that the occurrence of 'true' flushing was similar in the diabetics treated by diet alone and in those treated with oral agents. By contrast, almost all of the aspecific flushers had been on long-term treatment with oral agents.

Table 2 shows the prevalence of vascular lesions. Even though CPAF-negative and AF showed a slightly higher prevalence of severe lesions with visual loss there was no significant difference between the groups. Unlike CPAF-negative and AF, none of the CPAF-positive patients showed loss of visual acuity due to maculopathy in spite of the fact that 16% of them had more than five retinal lesions.

	CPAF positive (true flushers)	CPAF-negative (non flushers)	AF (aspecific flushers)	total flushers (CPAF-positive plus AF)
<i>retinopathy</i>				
nil/minimal	16 (84%)	67 (92%)	11 (69%)	27 (78%)
severe, no visual loss	3 (16%)	2 (3%)	1 (6%)	4 (11%)
severe, visual loss	0 (0%)	4 (5%)	4 (25%)	4 (11%)
<i>electrocardiogram *</i>				
normal	14 (73%)	39 (54%)	8 (50%)	22 (63%)
abnormal				
- rhythm or conduction defects	5 (27%)	25 (34%)	5 (31%)	10 (28%)
- perfusion alteration	0 (0%)	9 (12%)	3 (19%)	3 (9%)
<i>oscillography **</i>				
normal	14 (74%)	38 (52%)	6 (37%)	20 (57%)
abnormal				
- reduced	4 (21%)	26 (36%)	7 (44%)	11 (32%)
- abolished	1 (5%)	9 (12%)	3 (19%)	4 (11%)

* the prevalence of normal electrocardiograms is significantly higher in 'true' flushers than in AF and CPAF-negative patients ($p < 0.001$)

** the prevalence of normal oscillograms is significantly higher in 'true' flushers than in AF ($p < 0.002$) and CPAF-negative patients ($p < 0.005$)

Tab. 2 - Distribution of retinopathy, electrocardiographic and oscillographic abnormalities in CPAF-positive, aspecific flushers (AF), non-flushers (CPAF-negative) and pooled flushers. Men and women pooled together.

	CPAF-positive (true flushers)	CPAF-negative (non-flushers)	AF (aspecific flushers)
SBP (mmHg)	155 ± 27	150 ± 20	164 ± 33
DBP (mmHg)	88 ± 14	88 ± 12	90 ± 16
TG (mg/dl)	137 ± 57	150 ± 96	144 ± 73
CHOL (mg/dl)	277 ± 47	265 ± 59	271 ± 66
HDL-CHOL (mg/dl)	58 ± 16	50 ± 16	59 ± 15
VIII:C (%)	122 ± 32	116 ± 31	126 ± 42
VIII:Ag (%)	157 ± 54	184 ± 75	161 ± 63
fibrinogen (g/dl)	0.29 ± 0.07	0.31 ± 0.09	0.32 ± 0.06
β-TG (ng/ml)	34.3 ± 36.1	32.2 ± 31.7	30.1 ± 27.4

Tab. 3 - Blood pressure, serum lipids and hemostatic variables. The values shown are means ± SD. SBP = systolic blood pressure; DBP = diastolic blood pressure; TG = serum triglycerides; CHOL = total serum cholesterol; HDL-CHOL = serum HDL-cholesterol; VIII:C = Factor VIII coagulant activity; VIII:Ag = factor VIII-related antigen; β-TG = β-thromboglobulin.

ECG abnormalities were separated into rhythm or conduction defects, and perfusion defects (ischemia or previous myocardial infarction). Normal electrocardiograms were found significantly more frequently in the CPAF-positive group than either the AF or CPAF-negative patients ($p < 0.001$). None of the 'true' flushers showed perfusion defects.

Seventy-four percent of the 'true' flushers had normal leg oscillograms, compared to the 52% in the CPAF-negative group ($p < 0.005$) and 37% in the AF group ($p < 0.002$). Only one of 19 patients (5%) lacked a peripheral pulse in the CPAF-positive group, compared with 9 of 73 (12%) in the CPAF-negative group.

Table 3 lists the values for other parameters studied in relation to the CPAF test. We did not observe any significant differences among the patient groups in systolic and diastolic blood pressure, total and HDL-cholesterol, triglycerides, VIII:C, VIII:Ag, plasma β-TG or fibrinogen.

As far as metabolic control is concerned, the 'true' flushers showed a mean value of HbA1 of $9.06 \pm 0.67\%$ SEM, the CPAF-negative subjects of $9.09 \pm 0.38\%$ SEM, the AF of $9.8 \pm 1.08\%$ SEM. The differences among groups are not significant.

Table 4 showed the prevalence of lesions when considering women separately. In the CPAF-positive group the prevalence of women with nil/minimal retinal damage is very close to that observed in tab. 2 for the pooled data (86% *vs* 84%), and the same thing happens for normal electrocardiograms (73% *vs* 73%) and oscillograms (74% *vs* 73%).

DISCUSSION

CPAF has been reported to be a dominantly inherited trait in non insulin-dependent diabetics, associated with low prevalence of retinal, heart and peripheral arterial lesions^{3,20}. Recently, however, the specificity and the significance of the phenomenon have been questioned, since many positive re-

sponses to the CPAF-test have been found in normal subjects and in insulin-dependent diabetics¹⁷. Moreover, in different diabetic clinics, testing diabetics for CPAF has led to a wide range of results^{17,19,20,22}. In this series of 108 non-insulin-dependent out-patient diabetics, the total prevalence of flushers was 32%, but almost half of those (46%) were aspecific flushers, in that they also flushed drinking alcohol after placebo. The percentage of 'true' flushers (17%) is therefore much lower than that reported either by LESLIE *et al.*²⁰ (66%) or by FITZGERALD *et al.*¹² (33%). The high prevalence of maturity onset diabetes of the young ('Mason type' diabetes) in the population studied by PYKE²⁴ might at least partially account for his higher number of CPAF-positive patients. FITZGERALD *et al.*¹² did not test the flushers with placebo, so their positive cases might well include a number of aspecific flushers. It cannot be ruled out, however, that there are true differences in the incidence of the phenomenon, related to the different genetic backgrounds of the patients.

The importance of distinguishing CPAF-positive patients from aspecific flushers is emphasized by the different prevalences of vascular lesions found in our series of diabetics than in previous reports^{12,20}. When the patients who flushed with alcohol after chlorpropamide and those who also flushed after placebo were considered together, the prevalence of lesions in both large and small vessels was similar to that of non-flushers. However, when the aspecific flushers were eliminated and the prevalence of lesions was evaluated in the 'true' flushers, they had no loss of visual acuity due to diabetic ma-

	CPAF-positive	CPAF-negative	AF
<i>retinopathy</i>			
nil/minimal	13 (86%)	26 (87%)	9 (82%)
severe, no visual loss	2 (14%)	1 (3%)	1 (9%)
severe, visual loss	0 (0%)	3 (10%)	1 (9%)
<i>electrocardiogram</i>			
normal	11 (73%)	13 (44%)	4 (36%)
abnormal			
- rhythm or conduction defects	4 (27%)	12 (40%)	4 (36%)
- perfusion alteration	0 (0%)	5 (16%)	3 (28%)
<i>oscillography</i>			
normal	11 (73%)	15 (50%)	4 (36%)
abnormal			
- reduced	3 (20%)	10 (33%)	4 (36%)
- abolished	1 (7%)	5 (17%)	3 (28%)

Tab. 4 - Distribution of retinopathy, ECG and oscillographic lesions in CPAF-positive, aspecific flushers (AF), non flushers (CPAF-negative) in women considered separately. The prevalence of lesions in the different groups of women is nearly identical to that observed in women and men pooled together. This seems to rule out the possibility that the reduced prevalence of lesions in CPAF-positive group might be accounted for by the increased percentage of women in that group.

culopathy. In addition, the prevalences of abnormal electrocardiograms and oscillograms are significantly higher in non-flushers. These differences in the prevalence of severe lesions cannot be accounted for by obvious differences in the composition of the patient groups, because the mean age and duration of diabetes were not significantly different between 'true' flushers and non-flushers. The aspecific flushers had had slightly longer mean duration of illness, which may explain their higher prevalence of lesions. Apparently this group is not protected against complications, as compared to non-flushers.

This study of a large number of patients has also allowed us to evaluate the incidence in our population of a number of variables which might affect the outcome of the CPAF test. Recently, positive responses in the test have been attributed to previous chronic treatment with chlorpropamide, with a very low incidence in non insulin-dependent diabetics treated by diet alone¹⁰. None of our patients was previously treated with chlorpropamide, and the 'true' flushers were present equally in the two groups of patients on diet and in those treated with oral agents. Unlike PYKE²⁴, we failed to observe an increased frequency of family history of diabetes in CPAF-positive patients; the high occurrence of dominantly-inherited 'Mason type' diabetes in the population studied by Pyke might explain its higher prevalence.

We have also evaluated whether metabolic or hemostatic parameters behave differently in relation to the CPAF phenomenon. The association between high levels of triglycerides and cholesterol and a higher risk for developing macroangiopathy has been clearly demonstrated in diabetics^{1, 7, 14, 25}. Although the significance of hemostatic factors in diabetics is controversial, a number of studies has shown that β -TG⁶, VIII:C and fibrinogen⁸ and VIII R:Ag²¹ are increased in complicated diabetes. Therefore, the availability of a group of patients protected against the development of severe vascular lesions seemed to give us an opportunity to investigate the role of lipid and hemostatic variables in the generation of these complications.

Our observations, however, failed to shed any light on this point, because none of the lipid and hemostatic variables was significantly different in the groups of diabetics subdivided according to their behavior in the CPAF test. These findings suggest that either these variables are not primarily involved in the development of the complications, their abnormalities in diabetes being secondary to the metabolic derangement, or that the mechanism of protection in CPAF-positive diabetics works through other routes.

Glycosylated hemoglobin as far as it does represent the metabolic control did not show differences among groups. We would also like to stress the fact that HbA1 does not give any information about metabolic control during the several years of illness of our patients and could not account for differences in prevalence of lesions.

The observed differences in sex distribution with a female-increased percentage in the flushers (either aspecific or 'true' flushers) raise the question whether a sex-linked factor might explain the different distribution of lesions. In fact, we tried to consider separately the prevalence data obtained from the 'true' flusher women and to compare them with those obtained from non-flusher women without finding changes from the results obtained with the pooled data (tab. 4). It should be also considered that the aspecific flushers were prevailingly women (69%) and showed, nevertheless, increased

prevalence of lesions. We feel therefore that the possibility that the different prevalences of lesions are to be ascribed to sex distribution can be ruled out.

Finally, we are aware that other risk factors may be claimed for vascular lesions and among them smoking. Only 18 out of our 108 patients were smokers, quite equally divided in the different groups, and only 9 of them smoked more than 10 cigarettes per day. It was therefore impossible to discriminate a specific effect of smoke.

In conclusion, we confirm the lower prevalence of vascular lesions in the CPAF-positive patients when the aspecific flushers are eliminated by a placebo test. The discordant reports concerning the percentage of positive patients may be due to the lack of objective means for judging the positiveness or to differences in the populations of diabetics. The large scale epidemiological study and the unification of procedures recently announced by JEFFERYS et al.¹⁵ must therefore be completed before we can use the CPAF-test for genetic counseling and prognostic labeling in non insulin-dependent diabetics.

SUMMARY

One hundred and eight non insulin-dependent diabetics were tested for alcohol flushing after chlorpropamide administration (CPAF test). The overall prevalence of patients who flushed at the first challenge was 32%. However, nearly half of them still flushed after alcohol administration, when placebo was given instead of chlorpropamide, so that the prevalence of 'true' flushers was only 17%. Even though the distribution of retinal lesions was similar in 'true' flushers and in non flushers, severe loss of visual acuity was confined to the non flushers and aspecific flushers. The frequency of pathological ECG findings and of peripheral pulse reduction or abolition was significantly higher in the non flushers and aspecific flushers. Blood pressure, serum lipids and hemostatic parameters were similar in the two groups, and therefore do not explain the differences in prevalence of lesions. This study confirms the previous findings of a lower prevalence of large vessel lesions in flushers; however, the prevalence of 'true' CPAF phenomenon in our out-patient population appears to be much lower than previously reported.

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