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SHORT COMMUNICATION

Occupational exposure to anaesthetic gases and urinary excretion of D-glucaric acid

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In order to ascertain whether the urinary excretion of D-glucaric acid (DGA) might be a suitable biomarker of effect in monitoring workers exposed to anaesthetic gases, we measured DGA before and after an operating session (and, in some workers, before and after a 2-week vacation) in 229 workers of surgical units and in 229 controls. In the former, we also measured urinary levels of nitrous oxide (N_2O) and isofiurane after at least 4 h of exposure. For all subjects, information on age, smoking habits, daily intake of alcohol, coffee, and drugs, history of liver or kidney disease was collected. Study subjects were ranked according to: exposure (class 0: subjects not exposed; class 1: $N_2O < 27$ μg $l^{-1}$and isofiurane < 1 μg l^{-1}; class 2: $N_2O < 27$ μg $l^{-1}$and isofiurane > 1 μg l^{-1}; class 3: $N_2O > 27$ μg $l^{-1}$and isofiurane < 1 μg l^{-1}; class 4: $N_2O > 27$ μg $l^{-1}$and isofiurane > 1 μg l^{-1}); general habits; and DGA (two groups, below and above the arbitrary cut-off value of 3.5 mmol mol^{-1} creatinine). The relative risk of presenting high DGA excretion was estimated through the Odds Ratio (OR) and 95% Confidence Intervals (CI). In univariate analysis, ORs increased from class 1 (lowest exposure) to class 4 (highest exposure) and with increases in coffee and cigarette consumption. The ORs adjusted for sex, age, creatinine, and alcohol and coffee intake, conventionally 1.0 in the control group, were 0.68 (CI = 0.33–1.38), 2.68 (CI = 1.36–5.27), 2.68 (CI = 1.21–4.90) and 3.73 (CI = 1.51–9.18) respectively in exposure classes 1, 2, 3 and 4. By contrast, individual levels of DGA did not correlate with urinary concentrations of anaesthetic gases. Moreover, no significant differences in DGA levels were observed between urine samples taken before and immediately after a workshift, nor between samples collected before and after at least 2 weeks vacation. In conclusion, DGA excretion cannot be used as an individual biomarker of effect in workers exposed to anaesthetic gases. Since effects on hepatic function were not found at lower concentrations (exposure class 1), the currently adopted threshold limits (isofiurane: 1 μg l^{-1}; and N_2O: 27 μg l^{-1}) appear sufficiently protective.

Keywords: D-glucaric acid, anaesthetic gases, nitrous oxide, isofiurane, enzymatic induction, occupational exposure, biological monitoring.

Introduction

In laboratory animals, various anaesthetic gases administered in high doses alone or in mixture induced morphological and functional changes in liver (alteration endoplasmic reticulum, steatosis, nuclear pycnosis and microsomal enzyme induction) (Brown and Sagalyn 1974, Peduto *et al*. 1983, Plummer *et al*. 1986).

Alterations in the endoplasmic reticulum were also found in histological samples of liver taken from patients receiving anaesthetics during diagnostic

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laparatomies (Sindelar *et al*. 1982). Instead, liver enzyme induction was reported in patients receiving mixtures of nitrous oxide (N_2O) and halothane (Cousins *et al*. 1987) but not in patients treated with other halogenated anaesthetics (Jones *et al*. 1990).

An increase in urinary excretion of D-glucaric acid (DGA), an indirect tool of microsomal enzyme induction, has been found in hospital workers exposed to mixtures of gases, mainly isofiurane and N_2O (Franco *et al*. 1991, 1992, Scapellato *et al*. 1994).

D-glucaric acid is the terminal metabolite of the biotransformation of glucuronic acid. It is still not clear what mechanism links the production of DGA to enzyme induction: the metabolism of glucuronic acid also involves enzymes not belonging to the microsomal system; nevertheless urinary excretion of DGA is increased by many substances known to be inducers of microsomal enzyme activity (Aartes 1965) and DGA correlates well with contents of microsomal enzymes in liver tissue samples (Lecamwasam 1975). In fact some authors suggest the use of DGA as an indicator of effect in exposure to xenobiotics (Foé *et al*. 1986, Franco and Fonte 1994).

It therefore appeared to be interesting to evaluate the behaviour of DGA urinary excretion in a population of operating-theatre staff in a large hospital and in a reference population, in order to determine the infiuence of exposure to anaesthetic gases on DGA and its possible use as a biomarker of effect.

Materials and methods

Sample populations

The sample populations consisted of 229 subjects (119 male, 110 female), all exposed to N_2O and isofiurane in various surgery units, and a control group of 229 subjects (167 males, 62 females) selected from the administrative personnel of various public organizations, not exposed to anaesthetic gases or other hepatotoxic substances. Each subject was given a questionnaire in order to collect information on age, habits (smoking, intake of alcohol and coffee), intake of drugs, and history of liver or kidney disease. The quartiles of age, creatinine and coffee intake were identified, according to which the whole population was subdivided into four subgroups of roughly the same number of subjects. Since non-smokers made up more than 75 % and non-drinkers more than 60 % of the population, cigarette number, years of smoking, years since stopping smoking, grams of alcohol intake, and years of drinking were subdivided using preconceived boundaries: 0, 1–9, 10–19, ≥ 20 (or 0, 1–24, 25–29, ≥ 30 for alcohol consumption). Each subgroup was coded as 0, 1, 2, 3, going from the lowest to the highest, thus transforming intervals into polytomous variables.

Biological indicators

Urinary concentrations of N_2O and isofiurane (2-chloro-2-(difiuoromethoxy)-1,1,1-trifiuoro ethane) were measured on urine samples collected at the end of the operating session or after at least 4 h of exposure, using the headspace method on a gas-chromatograph equipped with an electron capture detector (Imbriani *et al*. 1988). Then, for N_2O, a biological exposure index (BEI) of 27 μg l^{-1} was applied, corresponding to the environmental italian limit of 50 ppm established for new or restructured operating-theatres by a Circular (n. 5/89) from the Italian Ministry of Health. For isofiurane, in the absence of environmental limits laid down in the above Circular, reference was made to a urinary concentration of halogenate of 1 μg l^{-1}, corresponding to the environmental threshold value of 0.5 ppm established by NIOSH in 1977 for simultaneous exposure to halogenated anaesthetics and N_2O. Assuming that the urine level of N_2O and isofiurane was zero in the non-exposed subjects, a polytomous exposure variable was built, which was 0 for control subjects and 1, 2, 3, 4 for those exposed respectively to: N_2O below 27 μg $l^{-1}$and isofiurane below 1 μg l^{-1}; N_2O below 27 μg l^{-1} but isofiurane above 1 μg l^{-1}; N_2O above 27 μg l^{-1} but isofiurane below 1 μg l^{-1}; N_2O above 27 μg l^{-1} and isofiurane above 1 μg l^{-1}.

In both exposed and control subjects, DGA was measured in urine samples collected at the beginning and end of a workshift. Analysis was carried out with a spectrophotometric method, and values are expressed as mmol according of mol of creatinine (mmol mol^{-1} Cr) (Colombi *et al*. 1983a). In addition, in 40 exposed subjects, urine samples were collected and analysed for DGA before and after a

period of vacation, far from exposure, lasting at least 2 weeks. To our knowledge, there are no published sources on the 'normal range' of urinary DGA. Assuming that DGA may only have an upper limit, we arbitrarily assessed as cut-off point the 75th percentile (= 3.5 mmol mol^{-1} Cr) which, among non-exposed subjects, divides the top 25 % with 'abnormal' from the 75 % with 'normal' DGA levels.

Statistical methods

Interval variables were analysed by common methods (Student's *t*-test, simple correlation). The dichotomous variable DGA was cross-tabulated with all other frequency variables, and the Odds Ratio (OR) and its 95 % Confidence Interval (CI) were then estimated (Breslow and Day 1987), by assuming at a conventional risk of 1.0 the group with the lowest value of the various indicators. For variables with naturally ordered classes, the chi-square test for trend (χ^2_{trend}) was also applied. In order to detect an association between DGA and exposure, holding constant possible confounders, stepwise multiple logistic regression analysis was used. A dichotomous variable (1 or 0 for subjects with DGA values above or below the cut-off) was the dependent variable; age, creatinine, coffee intake, number of cigarettes, years of smoking, years since stopping smoking, alcohol intake, years of alcohol drinking (all polytomous variables as above) and exposure groups (five classes) were independent variables. The coefficients of logistic regression, returned from logarithmic to a normal scale, are ORs, one adjusted for the effect of the others. 95 % CI were also provided by the program. Statistical analysis was carried out by means of the BMDP statistical package (Dixon 1992).

Results

Table 1 lists the main characteristics of the two populations. It may be seen that workers in surgical units are younger and drink less alcohol than controls. None of the study subjects had recently taken drugs known to interact with the mixed-function oxidase system, and none of them reported chronic liver or kidney disease.

Table 2 lists number of subjects, means, standard deviations, and range of urinary levels of anaesthetic gases found in the four classes of workers. Exposure to anaesthetic gases tended to be high. Out of 229, 118 workers had either N_2O or isofiurane above the corresponding BEIs (of 27 $\mu g\, l^{-1}$ for N_2O and 1 $\mu g\, l^{-1}$ for isofiurane), and 31 had values exceeding threshold limits for both gases.

Table 1 shows also the mean urinary concentrations of DGA (post-shift values throughout, unless otherwise specified) in exposed and control subjects, which substantially overlap (range = 0.6–12.8 mmol mol^{-1} Cr and 0.8–8.6 mmol mol^{-1} Cr respectively). High DGA levels in workers with concentrations of anaesthetics exceeding the threshold limits (table 2) may have been diluted by lower values in workers with compliant exposure. Additionally, there may have been a confounding infiuence of age and alcohol consumption, which is higher in controls (table 1).

Table 1. Mean values (*X*) and standard deviation (SD) of relevant variables in study groups.

Variables	Exposed group		Control group	
	X	SD	*X*	SD
Age	34.54	8.78	42.24	8.47
Coffee cups per day	2.30	1.82	2.34	1.36
Cigarettes per day	2.67	5.75	2.68	6.20
Smoking (years)	3.17	6.51	3.28	7.72
Alcohol (g per day)	6.92	15.59	22.20	25.88
Drinking (years)	3.28	14.22	11.87	11.96
Creatinine (gl^{-1})	1.90	0.91	1.68	0.76
DGA (mmol mol^{-1} Cr)	3.18	1.52	2.94	1.13

Table 2. Urinary concentrations (expressed in μg l^{-1}) of N_2O and isofiurane in exposed subjects.

Concentration	No. subjects	Mean	SD	Range
$N_2O < 27$ μg l^{-1}	90	11.43	8.87	0–26.9
Isofiurane < 1 μg l^{-1}		0.27	0.25	0–0.9
$N_2O < 27$ μg l^{-1}	65	4.47	6.63	0–19.8
Isofiurane > 1 μg l^{-1}		4.54	4.85	1.0–30.7
$N_2O > 27$ μg l^{-1}	43	73.58	61.42	27.2–342.4
Isofiurane < 1 μg l^{-1}		0.23	0.28	0–0.9
$N_2O > 27$ μg l^{-1}	31	116.31	42.42	27.4–189.1
Isofiurane > 1 μg l^{-1}		2.51	1.44	1.0–5.7

There was no statistically significant correlation in exposed subjects between DGA excretion and urinary levels of isofiurane ($y = -0.0019x + 3.3976$, $r = 0.0082$; n.s.) and N_2O ($y = 0.0323x + 3.1228$, $r = 0.0067$; n.s.).

In the classes of various risk indicators table 3 shows the distribution of subjects with low (normal) or high (abnormal) DGA values and the results of univariate statistical analysis. It can be seen that classes 3 and 4 with the greatest exposure have an OR of abnormal DGA, respectively about two and three times higher with respect to non-exposed subjects, with a progressive and highly significant increase in risk from class 1 to class 4 (chi-square test for trend). It emerged that, as well as exposure, intake of coffee, urinary creatinine level, cigarette consumption, and sex significantly infiuenced DGA excretion.

Table 4 shows how, holding constant all possible confounding factors (sex, age, creatinine, alcohol, coffee) by means of multivariate analysis, classes 2, 3 and 4 have significantly increased risks of having DGA urinary concentrations exceeding 3.5 mmol mol^{-1} Cr. Instead, class 1 (isofiurane and N_2O both below thresholds) had an OR of 0.68, not significantly different from the assigned value of 1.00 given, i.e. the risk of non-exposed subjects.

There were no statistically significant differences between mean urinary concentration of DGA at the beginning and at the end of workshift in exposed subjects (before the shift = 3.21 – 1.73 mmol mol^{-1} Cr; end of the shift = 3.18 – 1.52 mmol mol^{-1} Cr), and the same result was obtained when subjects were subdivided according to level of exposure to anaesthetic gases.

Lastly, in a smaller number of exposed subjects, no statistically significant differences were revealed between DGA urinary concentrations measured before and immediately after a vacation of at least 2 weeks (before vacation = 3.00 – 1.19 mmol mol^{-1} Cr; after vacation = 3.26 – 1.07 mmol mol^{-1} Cr).

Discussion

In the present study, the mean urinary excretion of DGA was not significantly different in exposed subjects and controls. However, when DGA values were dichotomized, subjects with exposure to one or both of the gases exceeding the BEIs were shown to be at high risk of having increased urinary excretion of DGA. These results were corrected for all possible interfering variables by uni- and multivariate analyses: urinary excretion of DGA is known to be an aspecific

Table 3. Numbers of subjects with abnormal or normal DGA values in classes of variables. Odds Ratio (OR) of abnormal DGA with 95% Confidence Intervals (CI) and chi-square trend test across classes of each variable.

Variable		Subjects with DGA				
	Class[a]	Abnormal	Normal	OR	CI	Trend test
Exposure						12.27*
	0	57	172	1.00		
	1	15	75	0.60	0.32–1.13	
	2	24	41	1.77	0.99–3.16	
	3	17	26	1.97*	1.01–3.87	
	4	15	15	3.02*	1.43–6.39	
Coffee (cups per day)						8.75*
	0	28	108	1.00		
	1	31	85	1.41	0.78–2.52	
	2	35	84	1.61	0.91–2.84	
	3	34	52	2.52*	1.40–4.56	
Creatinine (gl^{-1})						6.38*
	0	44	70	1.00		
	1	31	75	0.66	0.37–1.15	
	2	22	98	0.36*	0.20–0.64	
	3	31	86	0.57	0.33–1.00	
Cigarettes per day						3.47
	0	91	260	1.00		
	1	15	26	1.65	0.84–3.23	
	2	11	30	1.05	0.50–2.18	
	3	11	13	2.42*	1.07–5.47	
Years of smoking						0.88
	0	91	260	1.00		
	1	16	16	2.86*	1.41–5.80	
	2	11	30	1.05	0.50–2.18	
	3	10	23	1.24	0.57–2.71	
Age (years)						0.14
	0	35	82	1.00		
	1	28	90	0.73	0.41–1.30	
	2	32	84	0.89	0.51–1.57	
	3	33	73	1.06	0.60–1.87	
Years of drinking						0.23
	0	78	198	1.00		
	1	4	8	1.27	0.37–4.32	
	2	20	42	1.21	0.67–2.19	
	3	26	81	0.81	0.49–1.36	
Alcohol (g per day)						0.06
	0	78	198	1.00		
	1	13	32	1.03	0.51–2.07	
	2	18	47	0.97	0.53–1.78	
	3	19	52	0.93	0.52–1.67	
Sex						
	Males	67	219	1.00		
	Females	61	110	1.81*	1.19–2.74	

[a] For definition of classes, see Statistical Methods section.

* $p < 0.05$.

Table 4. Odds Ratio (OR) and Confidence Intervals (CI) of having abnormal urinary excretion of DGA (above 3.5 mmol mol^{-1} creatinine) in classes of exposed surgical workers with respect to that of control subjects.

Classes of exposure	OR[a]	CI
No exposure	1.00	–
$N_2O < 27$ μg l^{-1} Isofiurane < 1 μg l^{-1}	0.68	0.33–1.38
$N_2O < 27$ μg l^{-1} Isofiurane > 1 μg l^{-1}	2.68*	1.36–5.27
$N_2O > 27$ μg l^{-1} Isofiurane < 1 μg l^{-1}	2.68*	1.21–5.90
$N_2O > 27$ μg l^{-1} Isofiurane > 1 μg l^{-1}	3.73*	1.51–9.18

[a] Corrected for creatinine, sex, age, alcohol and coffee intake by logistic regression analysis.
* $p < 0.05$.

marker, in that it may be infiuenced by many environmental, biological and behavioural factors, which must therefore be carefully assessed when interpreting results (Colombi 1987). It emerged that the factors which infiuence the urinary excretion of DGA in a statistically significant way are not only exposure, but also consumption of coffee and cigarettes, as found by Colombi *et al*. (1983b). However, Bisio *et al*. (1993) also found that alcohol intake had a significant infiuence.

Our data also showed that there were no significant differences in the urinary excretion of DGA in subjects exposed before the beginning and end of the operating session, both total operating-theatre staff and subjects subdivided into exposure classes. In this sense, we could not confirm the reports of Franco and colleagues (1991, 1992, 1994) of increased urinary excretion of DGA in exposed subjects at the end of the operating session, i.e. an acute effect exerted by anaesthetic gases. Nevertheless Franco's results have been found in a small number of workers exposed to anaesthetic gases concentrations higher than in our study (up to 900 ppm N_2O and up to 10 ppm isofiurane). Nor could we find significant differences in excretion of DGA in urine samples from operating-theatre staff collected before and immediately after a 2-week vacation far from exposure.

Therefore, our study shows that there is an effect on liver function linked to exposure to N_2O and isofiurane in operating-theatre staff, since this effect was found after correction for all possible interfering variables. This appears to be a chronic effect which only takes place after exposure to quite high levels of anaesthetic gases, in any case exceeding the respective BEIs. These conclusions led to the following considerations:

1. The first regards which type of hepatic effect is linked to increased urinary excretion of DGA. Although many authors believe that DGA excretion is an indirect and non-invasive measure of enzyme induction, for the reasons mentioned in the Introduction, we believe that new studies should be planned to clarify the metabolic pathway which links enzyme induction with urinary excretion of DGA, and in particular to define if there is a cause–effect relationship or two separate, independent mechanisms, each intervening at the hepatic level during exposure to xenobiotics.

2. Secondly, the evidence of a wider range of DGA in exposed subjects rather than in controls (including more extreme values at the top of the scale in the former) indicate that occupational exposure to anaesthetic gases causes liver enzyme induction in some, not all, exposed subjects.

3. The third point concerns exposure threshold levels. It may be stated, on the basis of our results, that the currently adopted BEIs are generally sufficiently protective, since effects on hepatic function were not found at lower concentrations.

4. Lastly, no correlations between urinary concentrations of anaesthetic gases and individual levels of DGA were found, indicating that the latter cannot, in our opinion, be used as a biomarker of individual effect of exposure to anaesthetic gases.

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