SHORT COMMUNICATION

Serum angiotensin-converting enzyme, soluble E-selectin and laminin in subjects occupationally-exposed to volatile organic chemicals and mercury vapour

Alison J. Stevenson and Howard J. Mason

This study attempts to confirm previous findings of altered serum soluble E-selectin and laminin in workers exposed to volatile organic chemicals (VOCs) and further explore evidence of endothelial cell disturbances in workers exposed to VOCs and mercury vapour. Serum angiotensin-converting enzyme (ACE), soluble E-selectin and laminin were measured in a cross-sectional study of workers exposed to a small range of VOCs (n = 21), mercury vapour (n = 32) and a control group (n = 50). Both endothelial markers, ACE and E-selectin were significantly higher in mercury- and VOC-exposed workers. Laminin was significantly lower than controls in the VOC-exposed. These results confirm our previous initial findings in workers exposed to VOCs of endothelial disturbances and decreased serum levels of a constituent of basement membranes, namely laminin. In the mercury-exposed workers who had relatively high occupational exposure for the UK, there was also evidence of endothelial disturbances. The ubiquitous nature of endothelial cells and the underlying structural basement membrane associated with the vasculature means that it is impossible to state with any certainty the site of the action of these chemicals. However due to the ease of absorption of VOCs and mercury via the pulmonary system, and the metabolism of VOCs in the lungs themselves, it is probable that the initial site of the disturbances noted is in lung endothelia and associated basement membrane.

Keywords: *soluble E-selectin, laminin, hydrocarbons, volatile organic chemicals, endothelial cells, pulmonary system, basement membranes.*

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Introduction

Angiotensin-converting enzyme (EC 3.4.15.1) is a peptidylpeptide hydrolase found mainly on the luminal surface of vascular endothelial cells and cells derived from the monocyte-macrophage system (Beneteau-Burnat and Buadin 1991). High levels of this membrane-bound protein are located in pulmonary capillaries. Increases in serum angiotensin-converting enzyme (ACE) have been reported in pathologies stimulating monocyte cell lines such the granulomatous disease, sarcoidosis (Gronhagen-Riska 1979). ACE has also been found to be abnormally high in occupationally-caused pulmonary diseases, such as silicosis and asbestosis (Gronhagen-Riska 1979, Owczarek and Lewczuk 1991), in current underground coal-miners (Thompson *et al.* 1991), as well as in several non-pulmonary chronic diseases including diabetes and alcoholic hypertension (Lieberman and Sastre 1980, Lauta 1990, Yamada *et al.* 1991). These findings suggest that increased ACE may reflect lung macrophage activation, disturbances of the vascular endothelium in the lung or of the generalized endothelia. Recently the measurement of the soluble form of the vascular adhesion molecules, E-selectin, has been reported to be a specific marker of endothelial cell activation or damage (Gearing and Newman 1993). Disturbances of the vascular endothelium may be important given its role in the control of vascular tone, haemostasis, permeability, fibrinolysis and synthesis of growth factors (Vane *et al.* 1990).

In a previous study on possible mechanisms for the relationship between volatile organic chemical (VOC) exposure and renal damage, we found indications of disturbances to vascular endothelium and the underlying basement membrane (Stevenson *et al.* 1995). Damage to basement membranes supporting endothelial structures within the lung and subsequent autoantibody production have been reported as a mechanism for initiating glomerulonephritis (Nelson *et al.* 1990). We have attempted to confirm those findings in a separate VOC-exposed cohort, as well as studying a group of workers exposed to mercury vapour, which is readily absorbed through the lungs and has been associated with renal damage (Bennet *et al.* 1991). We report here ACE levels and serum soluble E-selectin in three groups of healthy individuals; two of the groups had been chronically exposed at work to either mercury vapour or a limited number of VOCs. Serum laminin, which relates to basement membrane turnover (Risteli *et al.* 1982, Tomono *et al.* 1991), was also measured.

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METHODS AND POPULATION

ACE was measured kinetically at 37°C using 1 mM 3-(2-furylacryloyl)-L-phenylalanylglycylglycine as substrate in pH 8.2 borate buffer. Serum-soluble E-selectin and laminin were measured using enzyme immunoassay (R & D Systems, UK). The antibodies used for the laminin assay are directed against two specific epitopes on the B2 chain of the laminin molecule, therefore both fragments containing these epitopes and the intact molecule are measured. Serum samples were stored at –70°C until analysed in randomized batches. Quality control samples were analysed with each batch of samples for each assay.

Mercury-vapour exposed workers (n = 32) were selected from volunteers participating in a large cross-sectional study of mercury exposure and health effects in the UK. Selected individuals had at the time of study both a blood mercury level of greater than 40 nmol l^{-1} and an untimed urinary mercury level of greater than 40 μmol mol^{-1} creatinine. Subjects had been occupationally-exposed to mercury vapour and not to any other heavy metal or VOC. Monitoring of atmospheric mercury exposure using passive sampling badges was performed on the same day as venepuncture and urine collection. The VOC-exposed subjects (n = 21) worked at a single factory where finished rubber and vinyl products were

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produced. Exposure was principally to toluene, *n*-heptane, methylcyclohexane and xylene. On the day of blood sampling the atmospheric levels for the four solvents were measured using personal diffusive sampling tubes (Wright 1987). An additive notional VOC exposure index was calculated for each subject calculated from fractions of the UK exposure limit for each VOC at the time of the study. None of the VOC- or mercury-exposed subjects had abnormal liver function tests (transaminases, gamma-glutamyl transferases (GGT), alkaline phosphatase, bilirubin) compared with laboratory reference ranges. Subjects in both occupational cohorts completed a short health questionnaire including questions on smoking and alcohol intake. The third group, controls ($n = 50$), were volunteers with no occupational mercury or VOC exposure. All individuals were healthy, without any history of diabetes, hypertension and not on any current prescribed medication.

Results

Table 1 shows both the measured personal exposure levels in the mercury- and VOC-exposed workers and mercury biological monitoring data. Table 2 shows the biochemical measurements, age, sex and current smoking status in the three groups. Serum ACE was significantly higher in subjects exposed to mercury vapour and VOCs. Soluble E-selectin was significantly higher in those subjects exposed to mercury vapour and was increased in the VOC-exposed group though this just failed to reach significance. Serum laminin was significantly lower in the VOC-exposed group. The VOC-exposed group had a significantly greater proportion of females when compared with the control group. When the influence of sex on the three biochemical measurements was investigated in the control group only ACE was shown to be statistically significantly higher in males than females (97 versus 79; $p = 0.047$). Current occupational exposure was higher in males than females as evidenced from blood mercury levels (mean 156 vs 85 nmol l^{-1}) and notional additive VOC exposure index (mean 0.44 vs 0.22). Table 3 shows the comparison of male VOC and mercury workers with control males.

The proportion of smokers and non-smokers was similar in all three groups (Table 2). Subdivision of the occupational cohorts and controls by current smoking status suggested that it had no significant influence on ACE, E-selectin or laminin levels.

A significant positive relationship between aclohol intake and serum-soluble E-selectin was found within the mercury workers ($r = 0.48$, $p = 0.005$) but not in the VOC-exposed subjects. After removal of those mercury workers with weekly alcohol intakes greater than the maximum value found in the other two groups (40 units per week), E-selectin levels were still significantly higher than controls ($p = 0.04$). Neither ACE nor serum laminin in the individual groups, or in combination, were related to alcohol intake. Positive correlation coefficients between ACE and E-selectin were found in both the VOC and mercury groups, namely 0.368 ($p = 0.13$) and 0.481 ($p = 0.008$) respectively.

Serum laminin was significantly lower in the VOC-exposed cohort compared with either the control or mercury-exposed group. Multiple regression analysis was performed using age, sex, alcohol intake, smoking status, duration of occupational exposure or an index of current atmospheric VOC exposure as

	Mean	Range
VOC exposure		
Toluene, ppm[a]	5	1–71
Xylene, ppm[a]	1.3	0.5–4
Methylcyclohexane, ppm[a]	9	4–29
n-Heptane, ppm[a]	9	4–28
Notional exposure index[a]	0.21	0.1–1.45
Exposure, years[a]	7.2	0.3–34
Mercury exposure		
Hg, mg m^{-3}	0.15	0.016–2.11
Urine, Hg, μmol mol^{-1} creatinine	59	41–134
Blood Hg, $nmol^{-1}$	104	48–293
Exposure, years[a]	5.2	0.6–47

Table 1. The means and ranges for VOCs and mercury vapour from the measured personal atmospheric samplers, expressed as 8-h TWAs, mercury biological monitoring data and duration of occupational exposure are shown.

[a] Geometric mean.

	VOC group $n = 21$	Hg group $n = 32$	Control group $n = 50$
ACE (IU l^{-1})	162 (49)	125 (65)	92 (30)
	$p < 0.01$	$p < 0.01$	—
E-selectin (μg l^{-1})	52.9 (15.9)	54. 4 (18.0)	44.3 (14.1)
	$p > 0.05$	$p < 0.05$	—
Laminin (μg l^{-1})	412 (78)	472 (62)	470 (87)
	$p < 0.05$	$p > 0.05$	—
Age (years)	36.6 (9.4)	40.5 (14.0)	43.7 (12.6)
	$p > 0.05$	$p > 0.05$	—
Sex (m/f)	8/13	26/6	35/15
	$p = 0.017$	$p = 0.31$	—
Current smoker (yes/no)	12/9	18/14	26/24
	$p > 0.05$	$p > 0.05$	—
Alcohol (units per week)	8 (1–37)*	12 (1–58)*	9 (0–40)
	$p > 0.05$	$p > 0.05$	—

Table 2. The means (sd) for the biochemical measurements in the three groups are shown, except for alcohol intake where median and range are shown.

Significance levels (p) between exposed and control groups were derived from Dunnett's multiple comparison test in one-way analysis of variance. Fisher's exact test was used to test significance of proportionality. Kruskal–Wallis test was used to compare alcohol consumption.

	ACE (IU l^{-1})	E-selectin (μg l^{-1})	Laminin (μg l^{-1})	Age (years)
VOC males	176 (46)	65.1 (18.4)	410 (72)	38.2 (9.6)
$n = 8$	$p < 0.01$	$p < 0.01$	$p < 0.05$	$p > 0.05$
Hg males	135 (60)	56.8 (17.6)	477 (63)	41.4 (14.4)
$n = 26$	$p < 0.01$	$p < 0.05$	$p > 0.05$	$p > 0.05$
Control males	97 (30)	46.1 (14.7)	480 (84)	43.0 (12.3)
$n = 35$	—	—	—	

Table 3. Comparison of biochemical measurements in male mercury and VOC workers with male controls.

p-Values were derived from Dunnett's multiple comparison tests from analysis of variance.

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explanatory factors for serum laminin levels in the VOC-exposed workers. None of these explanatory factors appeared as significant influences on serum laminin levels.

Discussion

The data in this report suggest that, at levels of occupational exposure encountered in the UK to either mercury vapour or some common VOCs, increased ACE and soluble E-selectin levels are found. The levels of blood and urine mercury found represent high occupational mercury exposure within the UK. A health guidance value of 20 μmol mol^{-1} creatinine for urinary mercury has recently been published (HSE EH40/96 1996). The finding for VOCs and soluble E-selectin confirms our previous observations in different VOG-exposed cohorts (Stevenson *et al.* 1995). However, for the mercury group the influence of alcohol consumption makes the effects of the metal exposure on increasing E-selectin levels a little less certain. None of the variables measured here were shown to be age-related.

Evidence for a decrease in serum laminin in a different group of VOC-exposed subjects has already been reported (Stevenson *et al.* 1995). We have found one clinical report of decreased, rather than increased, serum laminin levels, reportably reflecting a pathological depression in basement membrane turnover (Veijola *et al.* 1993). The data from a few other occupational VOC studies are inconsistent. A study by Mutti *et al.* (1992) described increased serum laminin levels in a cross-sectional study of workers exposed to perchloroethylene; whereas Hotz *et al.* (1993) found decreased serum laminin in workers exposed to hydrocarbons for a long time, ascribing this to a selection or 'healthy worker' effect. Whether some VOC exposures directly affect basement membrane metabolism merits further study.

ACE is located in high concentrations on the luminal surface of vascular endothelial cells, especially the lungs, and cells derived from the monocyte-macrophage system (Beneteau-Burnat and Buadin 1991). Whilst ACE has been used to diagnose sarcoidosis, it is increased in other pulmonary pathologies such as silicosis and asbestosis (Gronhagen-Riska 1979, Owczarek and Lewczuk 1991). Increased levels of ACE have also been reported in active underground coal-miners (Thompson *et al.* 1991); the authors suggested that ACE may reflect alveolar macrophage activation due to inhaled dust. However, ACE has been reported to be elevated in extra-pulmonary diseases including alcoholic hypertension and diabetes (Lieberman and Sastre 1980, Yamada *et al.* 1991), and there is wide interindividual variation of ACE even in normal subjects due to a large genetic component (Alhenc-Gelas *et al.* 1991). The VOC and mercury workers in this study had neither abnormal GGT activities, excessive alcohol intake nor a history of hypertension or diabetes. In none of the groups studied was reported current alcohol intake related to ACE levels.

There are presently less reported data on serum-soluble E-selectin levels in disease, pathology or toxicology. Increased levels of soluble E-selectin reflect inflammatory insults specifically to endothelial cells due to its restricted secretion from that cell type, being increased in diabetes, hypertension, vasculitis and scleroderma (Carson *et al.* 1993, Gearing and Newman 1993, Blann *et al.* 1994). The increased levels of both ACE and serum E-selectin in the VOC-exposed workers may reflect the chronic interaction of volatile VOCs with endothelial cells in pulmonary capillaries as the VOC transfers from the inhaled gaseous phase into intra- and extra-cellular fluids. Metabolism of solvents such as xylene and toluene to reactive metabolites occurs in lung tissue (Patel *et al.* 1978) and may lead to an inflammatory cellular response.

The poor level of correlation between serum ACE and soluble E-selectin suggests that these biomarkers reflect different aspects of endothelial cell disturbances or different sites of effect. Given the importance of the inhalation route for mercury vapour absorption and the rapid intracellular accumulation and oxidative metabolism of metallic mercury to the protein-binding mercuric form (Berlin 1986), it is not unlikely that high levels of inhaled mercury vapour could influence the status of pulmonary endothelial cells. Thus, the increases in ACE and soluble E-selectin in both the VOC and mercury workers represent a subclinical disturbance of the vascular endothelium, probably in the lungs.

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