Biochemical effects of vinyl chloride monomer on the liver of occupationally exposed workers

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الآثار الكيميائية الحيوية لكلوريد الفاينيل الموجود على الكبد في العمال المعرَّضين له مهنياً

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خلاصة: قمنا ببحث تأثيرات التعرُّض لكلوريد الفاينيل الموجود (مونومير) على الكبد، في 86 من العمال، وذلك بقياس مستويات بيتا غلوكورونيداز، وآريل سلفاتاز "أ"، ونازعة أمين الأدينوزين، و5' نيوكليوتيداز، وإنزيمات وظائف الكبد الروتينية في أمصال العمال. ولقد وجدنا أن ثلاثة أو أكثر من هذه المعالم مرتفعة في 21 عاملاً، مع انخفاض ملحوظ في مستوى الغلوتاثيون في الدم، وارتفاع ملحوظ في مستوى النشاط الإنزيمي لترنسفيراز الغلوتاثيون "إس". ومن بين هؤلاء العمال الواحد والعشرين كان لدى 14 ارتشاح شحمي بالكبد مع تضخم كبدي في ثمانية منهم. كما كان لدى أربعة من العمال تضخم كبدي من دون ارتشاح شحمي، وكان لدى ثلاثة تضخم بالطحال. ويتبين من هذه الدراسة أن هناك حاجة إلى اليقظة في رصد البيئة، والفحص الطبي للعمال المعرَّضين لهذا المركب الكيميائي.

ABSTRACT We investigated the effects of vinyl chloride monomer exposure on the liver of 86 workers by measuring β-glucuronidase, arylsulfatase A, adenosine deaminase, 5′-nucleotidase and routine liver function enzymes in the sera of the workers. In 21 of them, three or more of these parameters were raised, with a significant decrease in the level of blood glutathione and a significant increase in the enzyme activity level of glutathione S-transferase. Of these 21 workers, 14 had fatty liver infiltration, 8 of whom were also suffering from liver enlargement. Also, 4 workers had liver enlargement without fatty infiltration and 3 had enlarged spleens. The study highlights the need for vigilance in environmental monitoring and medical surveillance of workers exposed to this chemical.

Effets biochimiques du chlorure de vinyle monomère sur le foie des ouvriers exposés au niveau professionnel

RESUME Nous avons examiné les effets du chlorure de vinyle monomère sur le foie de 86 ouvriers en mesurant la ß-glucuronidase, l'arylsulfatase A, l'adénosine-désaminase, la 5′-nucléotidase et les enzymes usuels pour la fonction hépatique dans les sérums des ouvriers. Chez 21 d'entre eux, trois ou plus de ces paramètres étaient en augmentation, avec une diminution significative du niveau de gluthation sanguin et une augmentation significative du niveau d'activité enzymatique du glutathion S-transférase. Parmi les 21 ouvriers 14 avaient une infiltration graisseuse du foie, dont 8 souffraient également d'une hépatomégalie. En outre, 4 ouvriers avaient une hépatomégalie sans infiltration graisseuse et 3 avaient une splénomégalie. L'étude souligne la nécessité d'une vigilance avec une surveillance de l'environnement et une surveillance médicale des ouvriers exposés à cette substance chimique.

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Introduction

Epidemiological studies have identified many cases of vinyl chloride-induced angiosarcoma of the liver in people employed in the manufacture of vinyl chloride [*1*]. Increases in other tumours, including liver, brain, lung, thyroid, lymphatic tissue and skin have also been noted. However, a relationship has not been established between these tumours and vinyl chloride exposure [*2*].

There seems little doubt that vinyl chloride is mutagenic and carcinogenic as a result of its metabolism by microsomal mixed function oxidases (cytochrome P-450) to chloro-oxirane (chloroethylene oxide) [*3*]. This highly electrophilic epoxide is a potent mutagen when tested directly or when generated from vinyl chloride in the presence of an appropriate metabolizing system [*4*]. This observation lead to the present investigation of the biochemical effects of occupational exposure to vinyl chloride monomer (VCM) on the livers of employees of the Egyptian Petrochemicals Company.

Participants and methods

The study was carried out on 106 males ranging in age from 23 years to 41 years, which included a control group of 20 healthy participants with no occupational exposure. The 86 volunteer workers with exposure to VCM were categorized according to where they worked at one of the following four operational sections of the Egyptian Petrochemicals Company.

- Group 1: 25 workers from the chlorine production unit;
- Group 2: 18 workers from the VCM unit (thermal cracking section);
- Group 3: 26 workers from the VCM unit (VCM purification section);
- Group 4: 17 workers from the VCM polymerization unit.

All participants were free from liver diseases. Full clinical and laboratory investigations were carried out to exclude patients suffering from any liver involvement or schistosomiasis, fascioliasis and viral hepatitis. Environmental air sampling was carried out at the different sections of the VCM production process using automatic thermal desorption sample tubes [*5*]. Blood samples were collected from all participants for assay of the enzyme activities of adenosine deaminase (ADA) [*6*], arylsulfatase A (ASA) [*7*], β-glucuronidase (β-glu.) [*8*], 5′ nucleotidase (5′-NT) [*9*], and liver function enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [*10*], and alkaline phosphatase [*9*].

The data in all groups were compared by analysing the variance between the parameters studied [*11*]. The correlation coefficient between the concentration of VCM and enzyme activity levels was also studied [*12*]. Workers with a high elevation in three or more of the aforementioned biochemical parameters (suspicious group) were investigated by ultrasound examination [*13*], determination of alpha-fetoprotein (AFP) in serum [*14*], reduced glutathione (GSH) content in whole blood [*15*] and glutathione S transferase (GST) activity in serum [*16*]. The results of this group were analysed using the Student *t*-test.

Results

Workers' average exposure was approximately 1.96 parts per million (ppm) for a maximum of 7.5 years duration. The threshold limit value-time weighted average (TLV-TWA) for VCM is 1 ppm (according to the United States Department of Labor's Occupational Safety and Health Administration concentration limits for gases).

Results of routine liver function tests were within normal range in all groups of

workers except in Group 4 where alkaline phosphatase activity exceeded the upper normal limit. There was a positive correlation coefficient between the concentration of VCM exposure and the enzymatic activity levels of ADA, β-glu. and 5′-NT in the sera of workers (Table 1). Statistical analyses of the variance between groups (Table 2) revealed a significant increase in the enzymatic levels of ADA, ASA, β-glu. and 5′-NT in the sera of the four studied groups compared to the control group. There was a significant increase in the enzymatic activity level of ASA in Groups 2, 3 and 4 compared to Group 1. All workers in the suspicious group had AFP levels within the normal range (0–20 IU/mL) [*14*].

The *t*-test analyses (Table 3) revealed a significant increase in ADA, ASA, β-glu., 5′-NT and GST enzymes activity levels in the sera of workers in the suspicious group compared to controls. There was a significant decrease in the GSH content in whole blood of workers in the suspicious group compared to controls. Among the workers in the suspicious group, clinical and ultrasonographic examinations indicated that 14 workers had fatty liver infiltration, 8 of whom were also suffering from liver enlargement. Also, 4 workers had liver enlargement without fatty infiltration and 3 had enlarged spleen.

Table 1 Correlation between concentration of vinyl chloride monomer and enzyme activity

Enzyme	*r*-value
Adenosine deaminase	0.59*
Arylsulfatase A	0.25
β-glucuronidase	0.78*
5′ nucleotidase	0.806*

**Statistically significant.*

Discussion

The present study endeavoured to elucidate the mechanisms by which VCM exposure leads to hepatopathy induction. The results revealed a significant increase in the enzymatic activity levels of ADA, ASA, β-glu. and 5′-NT in the sera of the exposed groups compared to the control group.

It has been reported that the serum level of β-glu. reflects the degree of histological hepatic cell necrosis [*17*]. There was significant positive correlation between the β-glu. level and the degree of hepatic cell necrosis determined by histological observation. On the other hand, there was no statistical correlation between the transaminase activities and the degree of hepatic cell necrosis [*17*]. It has been confirmed by immunohistochemical study that the increased β-glu. in serum is released from necrotic hepatic cells into the blood stream [*17*]. It was speculated that the elevation of serum transaminase activities resulted from the alteration in the membrane permeability of hepatic cells rather than from hepatocellular necrosis [*17*]. These results suggest that the serial measurement of β-glu. could be used as an indicator to predict the histological progression of hepatitis [*17*].

It has also been observed that the activities of certain arylsulfatases vary tremendously in some pathological conditions [*18*]. Thus, elevated activities of these enzymes may indicate an inflammatory process, as it is known from other sources that organs with high metabolic activity [*19*] and proliferating cells [*20*] have characteristically high arylsulfatases activity [*20*].

In past years, there have been numerous reports from many countries demonstrating a significant excess of chromosomal aberrations among workers exposed to vinyl chloride [*21*]. The evidence for random distribution of spontane-

Table 2 **Intergroup comparison between the four different enzymes among the studied groups**

Enzyme	Normal control	Group 1	Group 2	Group 3	Group 4	*F*-value (*P*)	Least significant difference
Adenosine deaminase (U/L)							Control and other groups
Mean	503.90	778.90	735.24	736.95	725.27	6.25	
s	130.83	230.53	364.82	276.25	173.70	(< 0.05)	
$s_{\bar{x}}$	29.26	51.55	38.48	60.28	44.85		
Arylsulfatase A (U/L)							Control and other groups
Mean	21.85	33.10	27.53	20.29	32.59	10.25	
s	5.65	11.39	9.38	10.52	12.71	(< 0.05)	
$s_{\bar{x}}$	1.26	2.43	2.27	2.15	3.08		
β-glucuronidase (U/L)							Control and other groups
Mean	9.10	18.64	20.13	21.13	20.18	4.25	
s	2.73	8.33	10.17	7.20	5.96	(< 0.05)	
$s_{\bar{x}}$	0.61	1.78	2.63	1.47	1.45		
5´nucleotidase (IU/L)							Control and other groups
Mean	6.50	12.43	14.71	10.89	12.86	6.25	
s	5.96	8.93	9.35	6.25	6.50	(< 0.05)	
$s_{\bar{x}}$	1.45	1.86	2.50	1.47	1.74		

s = *standard deviation.*
$s_{\bar{x}}$ = *standard error of the mean.*

Table 3 Statistical analyses of the biochemical investigations for normal control subjects (NCs) and workers group of suspicious cases (SCs)

Measurement	ADA (U/L)		ASA (U/L)		β-glu. (U/L)		5′-NT (IU/L)		GST (IU/L)		GSH (mg%)	
	NCs ($n=20$)	SCs ($n=21$)	NCs ($n=20$)	SCs ($n=21$)	NCs ($n=20$)	SCs ($n=21$)	NCs ($n=20$)	SCs ($n=21$)	NCs ($n=10$)	SCs ($n=21$)	NCs ($n=10$)	SCs ($n=21$)
Mean	504.0	920.0	22.0	38.0	9.0	24.0	7.0	16.0	5.0	8.0	38.0	29.0
s	131.0	323.0	6.0	15.0	3.0	9.0	4.0	7.0	1.6	2.6	2.2	5.4
$s_{\bar{x}}$	29.0	76.0	1.0	4.0	0.6	1.9	0.8	1.5	0.5	0.6	0.7	1.2
t[a]	5.11	3.88	7.53	5.29	3.84	−6.48						
P[b]	< 0.001		< 0.001		< 0.001		< 0.001		< 0.001		< 0.001	

[a] *t-test for comparison between workers group of suspicious cases and controls.*
[b] *Probability value for comparison between workers group of suspicious cases and controls.*
ADA = adenosine deaminase. *β-glu. = β-glucuronidase.* *GST = glutathione S transferase.* *5′-NT = 5′ nucleotidase.*
ASA = arylsulfatase A. *s = standard deviation.* *GSH = reduced glutathione.* *$s_{\bar{x}}$ = standard error of the mean.*

ous breaks in human lymphocytes is well known from an extensive study by Funes-Cravioto et al. [*22*] who analysed spontaneous breaks in lymphocytes. ADA and 5′-NT in the purine salvage pathway are examples of enzymes that have been extensively investigated [*23*]. In addition to their abundance in lymphoid tissues, the levels of these enzymes might reflect the lineage of specific lymphocyte populations [*23*]. Studies have indicated that the levels of these enzymes correlate with conventional immunologic markers [*24*]. 5′-NT enzyme activity has been found to be higher in B cells than in T cells [*25*] and to correlate with cell maturity [*26*]. ADA is an enzyme also involved in the purine salvage pathway [*27*]. This observation may explain of the significant increase in enzyme activity levels of both ADA and 5′-NT in the sera of workers exposed to vinyl chloride in the present study.

Our study demonstrates that occupational exposure to vinyl chloride may damage the liver. Hepatic injury developed insidiously without liver-specific symptoms and no disturbances were observed in routine liver function examination. About 76% of the workers (65 workers) were asymptomatic and had a normal range of most of the parameters studied, while 24% (21 workers) (suspicious group) had abnormally high levels of three or more parameters.

The workers in this latter group had a significant decrease in the level of blood GSH content and a significant increase in the GST activity level. Thus GSH is probably the most important cellular antioxidant [*28*]. Cells deprived of GSH typically suffer severe oxidative damage associated with mitochondrial degeneration [*28*]. As noted before, VCM appears to be mutagenic and carcinogenic as a result of its metabolism by microsomal mixed function oxidases (cyto-

chrome P-450) to chloro-oxirane (chloroethylene oxide) [3]. This epoxide has been found to be a potent mutagen when tested directly or when generated from vinyl chloride in the presence of an appropriate metabolizing system [4]. The other known mutagenic metabolite of VCM is chloroacetaldehyde, the rearrangement product of chloro-oxirane (chloroethylene oxide) [29]. The major detoxification pathway for these two mutagenic metabolites is by conjugation with GSH [30]. The enzymes catalysing these reactions are called glutathione S-transferases and are present in high amounts in liver cytosole and in lower amounts in other tissues [31]. If the potentially toxic xenobiotics were not conjugated to GSH, they would be free to combine covalently with DNA, RNA, or cell protein and could thus lead to serious cell damage [32]. It has been reported that the blood level of GST [33] or ligandin [34] increases in human liver disease because it is assumed to escape from liver cells into the blood under pathological conditions [16]. Marked increase in serum GST is observed when there is severe degeneration or necrosis of liver cells at the time of blood collection [16]. The aforementioned observations are in line with our findings which indicate the increased enzyme activity level of GST in the blood of the suspicious group.

Clinical and ultrasonographic examinations in our study indicated that 14 workers had fatty liver infiltration, 8 of whom were also suffering from liver enlargement; 4 workers had liver enlargement without fatty infiltration and 3 had enlarged spleen. In a previous study, histopathological observation of the liver of workers exposed to VCM in low doses revealed reactive hepatitis, slight inflammatory cell infiltration [35], Kupffer cell activity and focal necrosis, with or without fat accumulation in the liver [35]. This offers a good explanation for the present biochemical results.

These observations highlight the need for continual vigilance with environmental monitoring and medical surveillance of VCM-exposed workers. The depletion of GSH content in whole blood levels associated with highly significant increases in the enzymatic activity levels of β-glu. and ASA persisting in the long term may severely affect workers' health. High levels of β-glu. and ASA indicate a predisposition to cancer that could be due to the liberation of the active carcinogens from glucuronide and sulfate conjugates. The diagnostic criteria of chemical hepatic injury are therefore proposed to alert medical professionals, industrial hygienists, safety personnel and factory inspectors in order to avoid or delay early occupational liver diseases.

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