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ENZYMATIC PROFILE OF THE LUNG LAVAGE FLUID: FURTHER BIOCHEMICAL EVIDENCES OF A TOXICITY TOLERANCE THRESHOLD FOR CHRYSOTILE ASBESTOS

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ENZYMATIC PROFILE OF THE LUNG LAVAGE FLUID: FURTHER BIOCHEMICAL EVIDENCES OF A TOXICITY TOLERANCE THRESHOLD FOR CHRYSOTILE ASBESTOS

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Although some epidemiological studies on asbestos workers seem to point out an overall linear dose-response relationship between cumulative exposure and the incidence of asbestos-related lung diseases, this linear relationship appears somewhat controversial as far as chrysotile exposure is concerned. To examine this relationship, the conscious sheep model was selected.

Canadian chrysotile (UICC B) was repeatedly administered in saline by intra-tracheal instillation. The animals were divided in a low dose (LD) exposure group (<500 mg total dust) and a high dose (HD) exposure group (<5000 mg total dust), while a third group of sheeps received only the saline vehicle (control). Following the 12 months-exposure period, the animals were studied further during four months to assess the evolution of the disease. The following enzymatic activities were measured on broncho-alveolar lavages (BAL) collected regularly during the study: lactate dehydrogenase, acid phosphatase, alkaline phosphatase and β-N-acetyl-glucosaminidase.

The results show that, throughout the exposure period, only the HD-exposure group presented steady and significant increases of all four BAL enzymes. During the four months following the last exposure to the chrysotile fibers, again, only the HD-exposure group presented significantly elevated enzyme levels in the BAL. Overall, the LD-exposure group showed only transient elevations of some of the enzyme activities, mainly at the beginning of the exposure period. In conclusion, we believe that these biochemical data are suggestive of a tolerance threshold in regard to the toxicity of chrysotile asbestos fibers.

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Introduction

Some epidemiological studies on asbestos workers have proposed a linear dose-response relationship between asbestos cumulative exposure and the incidence of asbestos-related lung diseases[1-3]. This was suggestive to some that there is no safe limit for asbestos exposure[4]. However, many other epidemiological studies do not concur for such linear dose-response relationship, especially as far as chrysotile exposure is concerned.

For example, Weill et al.[5] report no evidence of a dose-response relationship in chrysotile cement workers. Similarly, McDonald et al.[6], found little or no excess risk of cancer or asbestosis in workers from a friction material plant. In a follow-up study of chrysotile mine and mill workers with the highest exposures, Becklake et al.[7] found no relationship between cumulative exposure indices and parameters of pleuropulmonary abnormalities. This study prompted these investigators to point out that a linear relationship had been previously proposed because an excessive number of heavyly exposed workers, in comparison to the number of low exposure workers, were included in the analysis[8]. Furthemore, a study of Canadian chrysotile miners exposed for 20 years to low levels of dust did not revealed an excessive prevalence of radiographic indices of lung diseases, findings that are in agreement with other studies from Italy and the Soviet Union[9-11]. More recently, Bégin et al.[12-14] showed no relationship between cumulative exposure indices from chrysotile workers and parameters of early or established asbestosis. These investigators were lead to believe that a cumulative index may not be the best parameter to use with chrysotile asbestos-related diseases.

Thus, substantial epidemiological data are available to support the concept of a tolerance threshold for chrysotile exposure. In this report, we wish to extend further the experimental evidences that, in the sheep model, the intensity and the rate of exposure are two important parameters to relate with the development of lung damage induced by chrysotile asbestos fibers[15].

Material and methods

The animals (male sheeps 20-40 kg) used in this study were the same as those described in earlier studies[16-19]. A pre-exposure study of the bronchoalveolar lavages (BAL) parameters did not show any significant variations between the animals[16]. Briefly, the method used to expose concious sheeps to chrysotile asbestos involved the nasotracheal intubation of UICC Canadian chrysotile fibers (UICC B, described by Timbrell[20]) resuspended in 50 ml of phosphate-buffered saline (PBS). Control sheeps received only the PBS solution. The chrysotile-exposed sheeps were divided in two categories: a) low doses (LD): this group included sheeps which had received between 412 and 424 mg of UICC B chrysotile asbestos during a 12 months-exposure period; b) high doses (HD): comparatively, this group received between 4244 and 4384 mg of asbestos fibers during the same period. During the first 5 months of the study, all the animals were injected intratracheally an average of 14 times; the single doses administered varied between 2 and 16 mg for the LD group and, between 1 and 128 mg for the HD group. During the 6th month following the first exposure to the asbestos fibers, supplementary doses of chrysotile (105 to 128 mg) were administered to bring the average cumulative doses of the LD group between 240 and 250 mg, and for the HD group between 1220 and 1440 mg. Thereafter, the difference in dosage between the two experimental groups was maintened by injecting the LD and

HD groups with 2 and 128 mg of UICC B chrysotile, respectively. The exposure parameters are briefly summarized in Table I.

The BAL effluents recovered from the sheeps were immediately spun down to recover the free cells. The cell-free supernatants were analysed on a Beckman Trace III Clinical Chemistry System equiped with a model 35 spectrophotometer. The enzyme activities, lactate dehydrogenase (LDH), alkaline phosphatase (AKP), acid phosphatase (ACP) and β-N-acetylglucosaminidase (β-NAG) were determined accordingly[21-23]. While for the AKP, ACP and β-NAG, one enzymatic unit express nmoles of p-nitrophenol released/min of incubation, for the LDH, one enzymatic unit express milli International Units (mIU)[22,23]. The experimental data were evaluated for statistical significance according to Tallarida and Murray[24]. When the F-values of the one-way analyses of variance were significant, the differences between the mean values of the experimental groups were compared accordingly (for the details on the selection of the appropriate comparison tests, see each figure legend).

Results and discussion

The present study show that increased levels of cytoplasmic (LDH)[21], lysosomal (ACP, β-NAG)[23] and lung surfactant (AKP)[25] -associated enzymes are detected in the airways of sheep exposed to chrysotile asbestos (Figures 1 to 4). However, the data clearly demonstrate that: A) only the sheeps exposed to high doses of chrysotile fibers presented significant elevations of all four parameters of the BAL fluid, when compared to the low dose group or the control sheeps; B) the responses of the high dose group show linear (ACP, β-NAG) (Figures 3 and 4) or near linear (LDH, AKP) (Figures 1 and 2) increases with the number of doses administered; C) that even 4 months after the end of the 12 months-exposure period, again, only the sheeps exposed to high doses of chrysotile fibers presented significant and sustained elevations of the four enzymatic activities (Table II).

The use of BAL fluid as an indicator of toxicity is well documented for lung targeted soluble and particulate materials[17-19,26,27],and high LDH, AKP and lysosomal activities have been associated with early and advanced asbestosis in humans[28,29]. These data provide substantial experimental evidences that there is a tolerance threshold level to chrysotile exposure, a concept that was recently reviewed by Dunnigan [30]. Moreover, this level cannot be considered only in terms of cumulative exposure but must take into consideration the intensity and the rate of exposures[15,31].

Although these biochemical data can be primarly associated with inflammatory activities initiating activation and liberation of these enzymes at the site of disease activities, the alveolitis is believed to be the key element to interstitial lung disorders, including asbestosis[32-34]. Therefore, since the induction of alveolitis by chrysotile asbestos seems to be dose-dependant[19,29], one must consider that there is indeed an exposure level below which no adverse health effects can be detected.

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Table I Exposure parameters.

Months of exposure		4	7	12
Number of exposure periods combined for analysis		3	2	1
Mean number of doses administrated		10	21	40
Mean cumulative amount of chrysotile administered (range)	LD	154 mg (28-240)	383 mg (382-384)	416 mg (412-424)
	HD	991 mg (580-1440)	2347 mg (2244-2464)	4315 mg (4292-4384)
Range of cumulative amount of chrysotile administered at the beginning of the exposure period	LD	10-96 mg	376-378 mg	412-424 mg
	HD	68-288 mg	1860-2080 mg	4292-4384 mg

LD: Sheeps exposed to low doses of chrysotile
HD: Sheeps exposed to high doses of chrysotile

Table II Enzymatic profile of the lung lavage fluid of sheeps post-exposure to chrysotile asbestos.

Exposure periods	Experimental groups	Number of animals	Enzyme activities (Mean value ± S.E.)			
			LDH (mIU/ml)	AKP (units/ml)	ACP (units/ml)	β-NAG (units/ml)
End of the 12 months-exposure period	C	6	9.12 ± 1.40	7.47 ± 2.39	0.61 ± 0.03	1.80 ± 0.18
	LD	5	14.12 ± 1.39	15.37 ± 6.73	0.87 ± 0.12	2.13 ± 0.21
	HD	6	46.22 ± 3.59*+	28.54 ± 11.09*	2.09 ± 0.20*+	6.89 ± 1.54*+
Post-exposure period (2 months)	C	6	7.08 ± 1.50	6.19 ± 2.16	0.51 ± 0.08	1.70 ± 0.26
	LD	5	10.68 ± 1.22	11.14 ± 2.63	0.64 ± 0.13	1.75 ± 0.33
	HD	5	33.32 ± 8.35*+	38.90 ± 9.92*+	1.98 ± 0.26*+	5.91 ± 1.77*+
Post-exposure period (4 months)	C	4	4.28 ± 0.66	3.75 ± 0.91	0.49 ± 0.06	1.60 ± 0.33
	LD	4	10.68 ± 2.92	12.22 ± 5.51	0.68 ± 0.19	1.77 ± 0.49
	HD	4	36.28 ± 10.46*+	28.72 ± 11.74*	1.83 ± 0.51*+	5.17 ± 2.12*

C: Control sheeps
LD: Sheeps exposed to low doses of chrysotile
HD: Sheeps exposed to high doses of chrysotile

* $p \leq 0.05$ Dunnett's multiple comparison test (one-way): C vs LD and HD
\+ $p \leq 0.05$ Students' t-test (one-way): LD vs HD

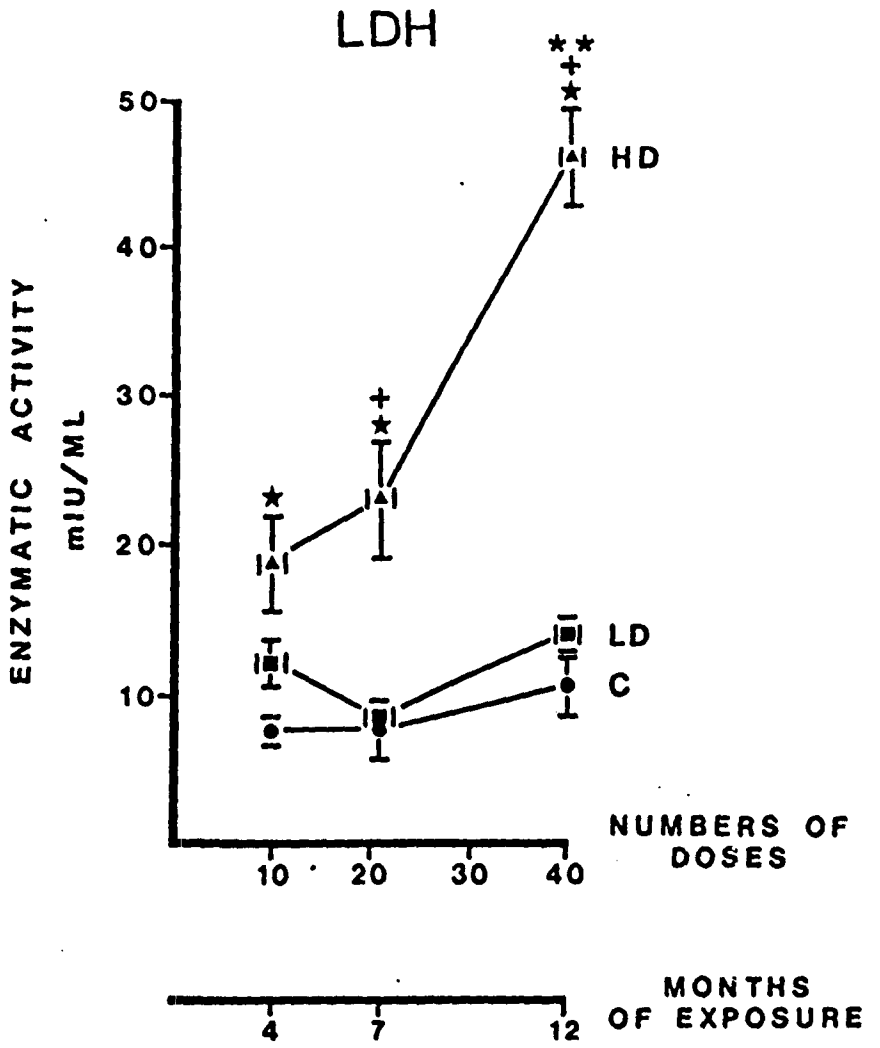

Figure 1 Enzymatic profile of the lung lavage fluid of sheeps exposed to chrysotile asbestos: Lactate dehydrogenase (LDH)

C: Control sheeps
LD: Sheeps exposed to low doses of chrysotile
HD: Sheeps exposed to high doses of chrysotile

Vertical bars: Standard error (S.E.) of the mean for the enzyme activities (n = 5 or 6)

Horizontal bars: S.E. of the mean for each number of exposure

★ $p \leq 0.05$ Dunnett's multiple comparison test (one-way)
C vs LD and HD

\+ $p \leq 0.05$ Students' t-test (one-way)
LD vs HD

** $p \leq 0.05$ Analysis of variance (one-way)
HD (12 months) vs HD (4 and 7 months)

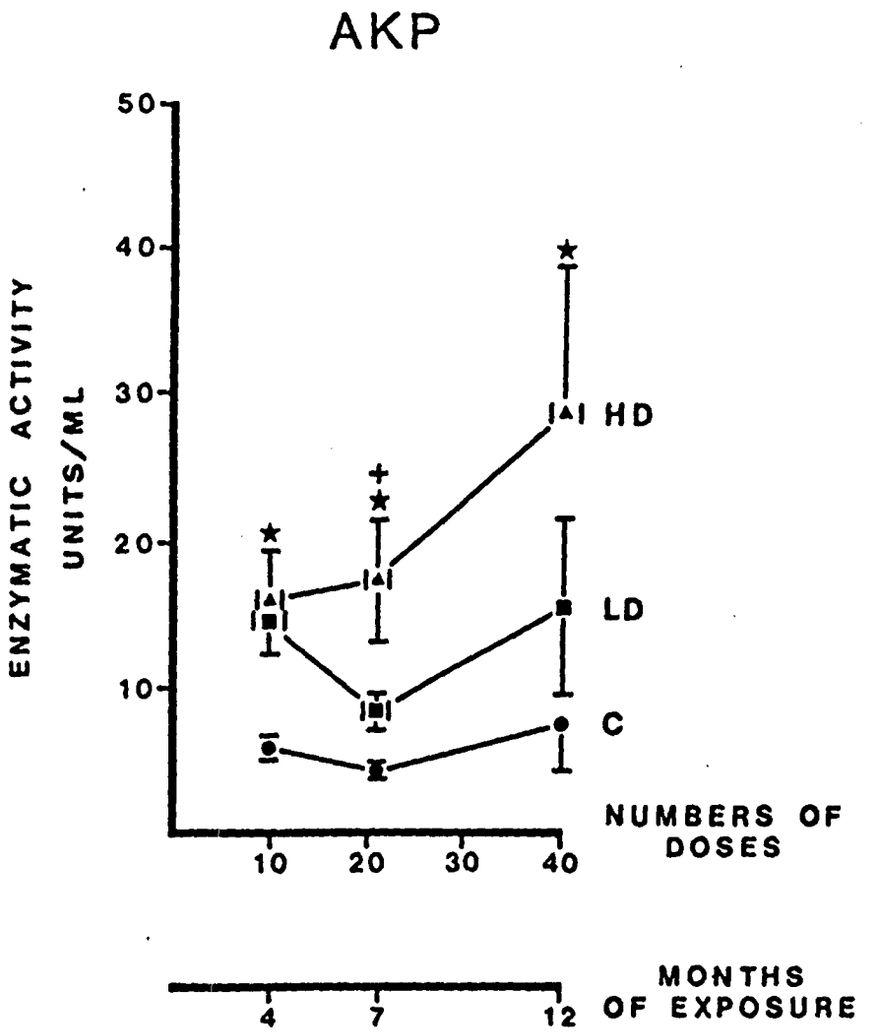

Figure 2 Enzymatic profile of the lung lavage fluid of sheeps exposed to chrysotile asbestos: Alkaline phosphatase (AKP)

C: Control sheeps
LD: Sheeps exposed to low doses of chrysotile
HD: Sheeps exposed to high doses of chrysotile

Vertical bars: Standard error (S.E.) of the mean for the enzyme activities (n = 5 or 6)

Horizontal bars: S.E. of the mean for each number of exposure

★ $p \leq 0.05$ Dunnett's multiple comparison test (one-way) C vs LD and HD

\+ $p \leq 0.05$ Students' t-test (one-way) LD vs HD

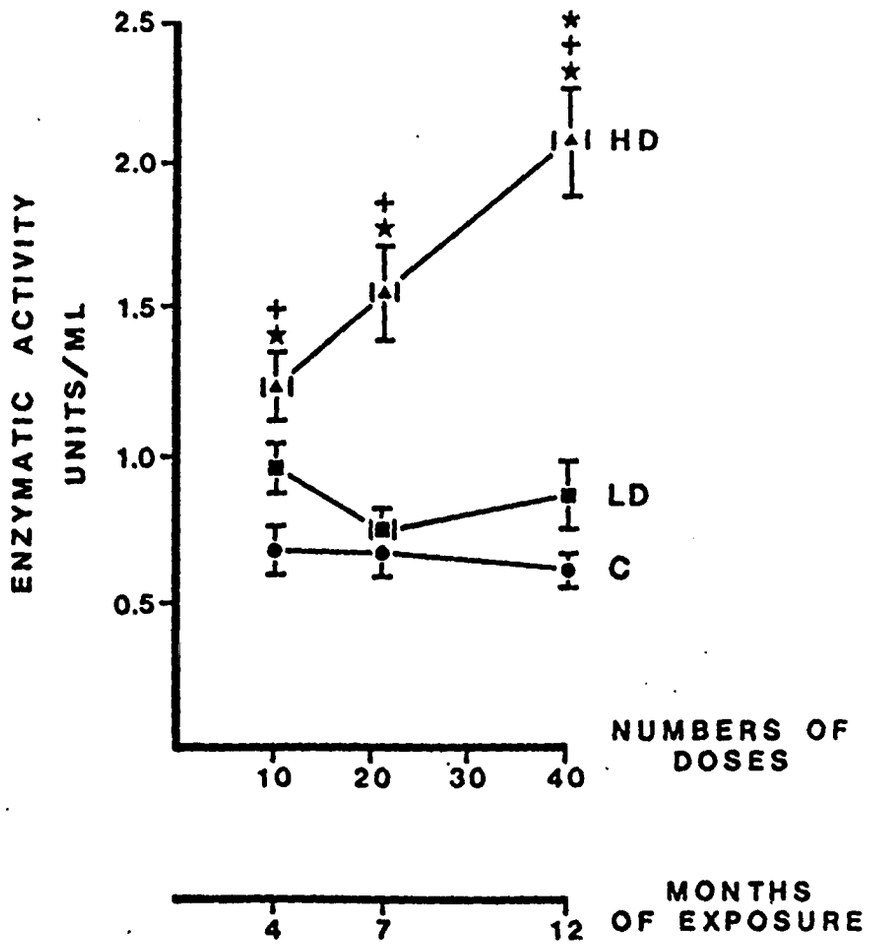

Figure 3 Enzymatic profile of the lung lavage fluid of sheeps exposed to chrysotile asbestos: Acid phosphatase (ACP)

C: Control sheeps
LD: Sheeps exposed to low doses of chrysotile
HD: Sheeps exposed to high doses of chrysotile

Vertical bars: Standard error (S.E.) of the mean for the enzyme activities (n = 5 or 6)

Horizontal bars: S.E. of the mean for each number of exposure

★ $p \leq 0.05$ Dunnett's multiple comparison test (one-way)
C vs LD and HD

+ $p \leq 0.05$ Students' t-test (one-way)
LD vs HD

* $p \leq 0.05$ Analysis of variance (one-way)
HD (12 months) vs HD (4 months)

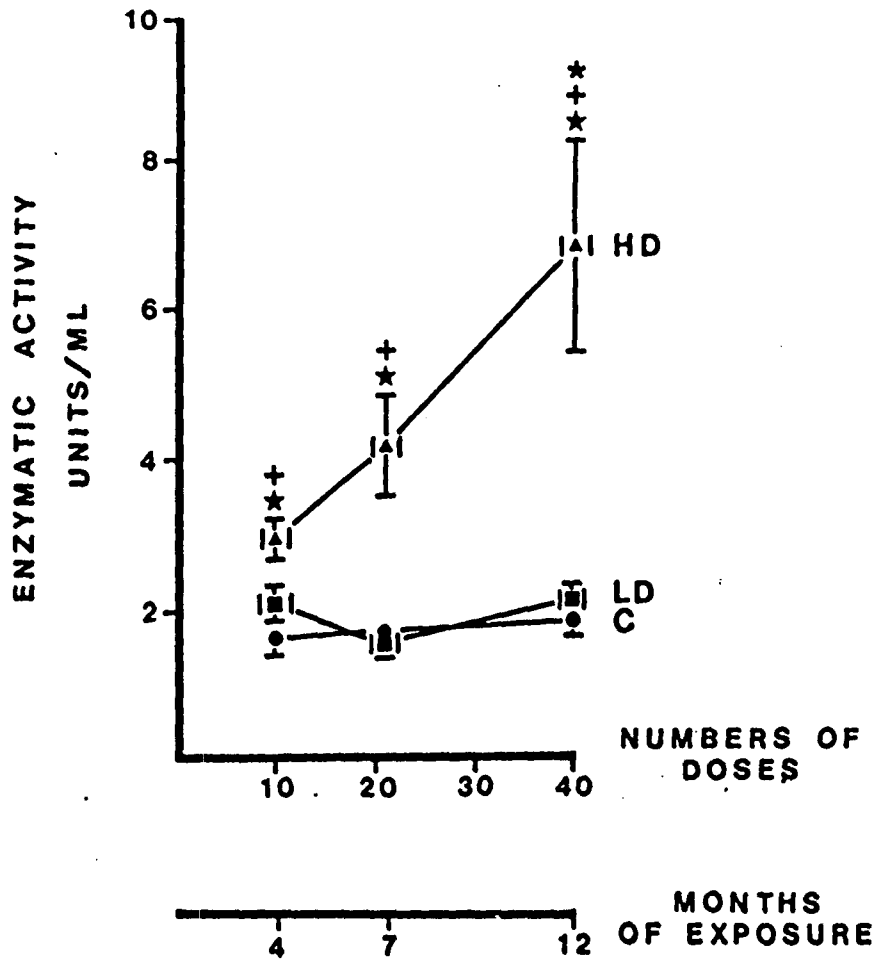

Figure 4 Enzymatic profile of the lung lavage fluid of sheeps exposed to chrysotile asbestos: β-N-acetylglucosaminidase (β-NAG)

C: Control sheeps
LD: Sheeps exposed to low doses of chrysotile
HD: Sheeps exposed to high doses of chrysotile

Vertical bars: Standard error (S.E.) of the mean for the enzyme activities (n = 5 or 6)

Horizontal bars: S.E. of the mean for each number of exposure

★ $p \leq 0.05$ Dunnett's multiple comparison test (one-way) C vs LD and HD

+ $p \leq 0.05$ Students' t-test (one-way) LD vs HD

★ $p \leq 0.05$ Analysis of variance (one-way) HD (12 months) vs HD (4 months)