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In Vitro Cytotoxic Effects of Wollastonites on Rat Hepatocytes: II. Lipid Peroxidation and Glutathione Depletion

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Hepatocytes have proven to be a useful model for studies of xenobiotic transformation (Croci and Williams, 1985), carcinogenesis, mutagenesis (Mitchell et al. 1983) and cytotoxicity (Chao et al. 1988, Sandy et al. 1988). They have also been used for the evlauation of cytotoxicity (Fleury et al. 1983, Denizeau et al. 1985a), carcinogenicity (Rahman and Casciano, 1985) and genotoxicity (Denizeau et al. 1985b) of asbestos and other silicate dusts. The superiority of these cells rests mainly upon their epithelial characters and well developed metabolic activation mechanisms. Since they seem to retain many of the essential properties of intact tissue including similar permeability characteristics. Therefore, in the present paper hepatocytes were selected as model system to study the comparative cytotoxic potential of wollastonite samples viz. kemolit ASB-3, kemolit-N and kemolit A-60 and chrysotile an asbestos form.

MATERIALS AND METHODS

Collagenase type IV, ethylene glycol bis-N,N,N,N',-tetraacetic acid, trypan blue and α-tocopherol were procured from Sigma Chemical Company, St. Louis, U.S.A., 2-thiobarbituric acid from B.D.H. Chemical Company, England. Other chemicals were purchased from either Glaxo Laboratories Bombay or Sisco Research Laboratory, Bombay, India, and were used without further purification.

Dusts. Wollastonite samples were provided by the Director, Wolkem Pvt. Ltd., Udaipur and were mined in Belkapahar in Tehsil Pindwara of District Sirohi, Rajasthan, India in crude form. Chrysotile was obtained from Andhara Pradesh Mining Corporation Ltd.,

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Hyderabad, and mined from Cuddapah, Andhara Pradesh, India. The particle size used, below 30 u, was prepared by the method of Zaidi (1969).

Male albino rats (200-250 g) obtained from ITRC animal house were maintained on diet supplied from Hindustan Lever Ltd., India and water ad libitum. Hepatocytes were isolated essentially by the method of Moldeus et al. (1978). All perfusion media were gassed with O_2/CO_2 (95%/5%) and maintained at 37°C. The portal vein of anesthetised rats was cannulated and the liver initially perfused with Hank's bicarbonate buffer (Ca free, pH 7.4) followed by collagenase (0.12% collagenase and 4 mM Ca) supplemented perfusion medium to digest the connective tissues at the flow rate of 15 ml/min for 6 min. The integrity of plasma membrane was evaluated by the cellular exclusion of trypan blue. The viability of the cells was 85-90% throughout the study.

Cells were suspended at the final concentration of 1 x 10^6 cells/ml.Cell suspensions were incubated with 100 ug dust samples at 37°C in a metabolic shaker with moderate shaking (80 oscillations/min). Aliquotes were taken at different time intervals and estimations were carried out.

Malonaldehyde (MDA) formation was measured in control and treated hepatocyte suspensions by the procedure of Wright et al. (1981). One ml of aliquot was taken and reaction was terminated by the addition of 0.3 ml 5 N HCl and 1 ml 10% trichloroacetic acid (TCA). Mixture was shaken and 1 ml of 2% thiobarbituric acid (TBA) was added. It was incubated at 90°C for 20 minutes, cooled in ice. Centrifuged at 2000 rpm for 10 minutes. Pink colour was read at 535 nm against a blank using spectronic 2000 spectrophotometer (Bausch and Lomb).

Intracellular GSH content was estimated in the cell pellets of control and treated hepatocytes by the method of Sedlack and Ludsay (1968). Cells were lysed with 0.1% EDTA solution and precipitating reagent which contains 0.16% metaphosphoric acid, 0.02% EDTA and 3% NaCl. After mixing, the solution was allowed to stand for five minutes before being filtered. Two ml of filtrate was added to 4 ml of disodium hydrogen phosphate (0.1 M, pH 8.0) and 1 ml of DTNB reagent (40 mg of 5,5'-dithiobis (2-nitrobenzoic acid is dissolved in 100 ml of 10 g/litre sodium citrate). A blank was prepared from 1.2 ml of precipitating reagent, 0.8 ml of EDTA solution, 4 ml of disodium hydrogen phosphate, and 1 ml of DTNB reagent. The colour of immediately read at 412 nm with the help of Spectronic 2000 spectrophotometer.

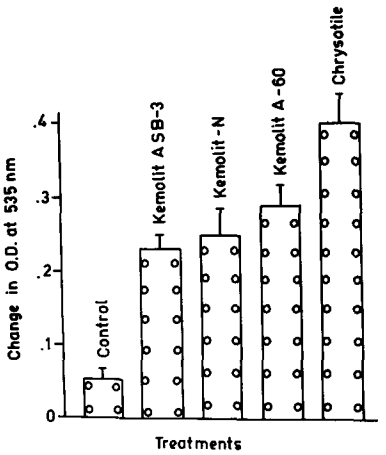

Figure 1. Kinetics of lipid peroxidation in control and dust treated hepatocytes

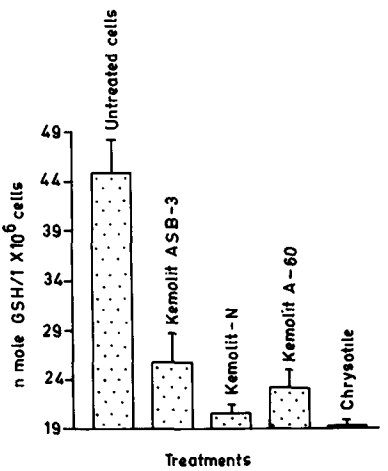

Figure 2. Kinetics of glutathione (GSH) content in control and dust treated hepatocytes

Values represent mean ± SE of six determinants. Experiments were repeated four times.

RESULTS AND DISCUSSION

A significant increase in malonaldehyde (MDA), TBA-reactant species, formation was observed in hepatocytes incubated 3 hrs with wollastonites and chrysotile (figure 1). Less lipid peroxidation (LPO) occurred when hepatocytes were incubated with wollastonites than with chrysotile. peroxidation was lower by 38, 40 and 49% for kemolit A-60, kemolit-N and kemolit-ASB-3, respectively. Chrysotile was the most toxic among the

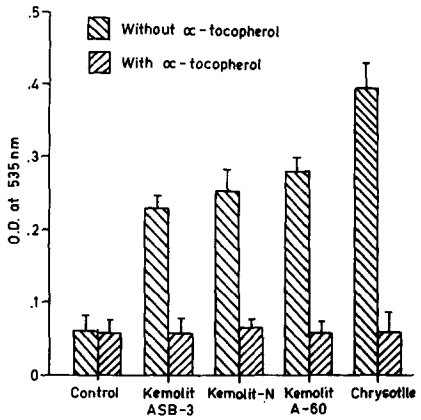

Figure 3. Effect of α-tocopherol on lipid peroxidation in control and dust treated hepatocytes.

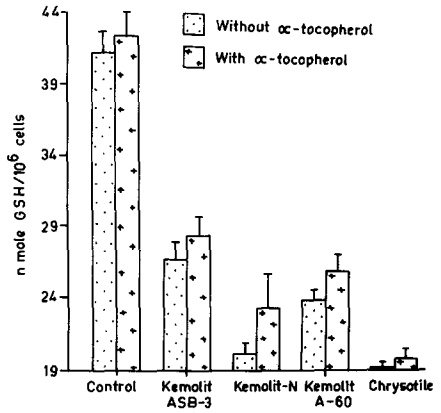

Figure 4. Effect of α-tocopherol on GSH control in control and dust treated hepatocytes.

evaluated samples and the results are in agreement with previous studies using human erythrocytes (Kennedy et al. 1989).

In contrast to lipid peroxidation (LPO), the intracellular glutathione (GSH) content was depleted to a significant degree in treated hepatocytes than in the control at 3 hr of incubation (Figure 2). The chrysotile asbestos was more prone to deplete GSH as compared

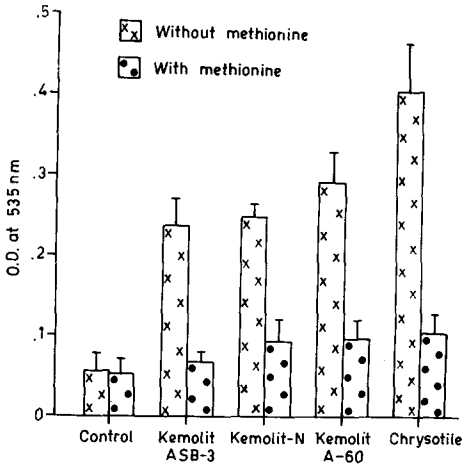

Figure 5. Effect of methionine on lipid peroxidation in control and dust treated hepatocytes.

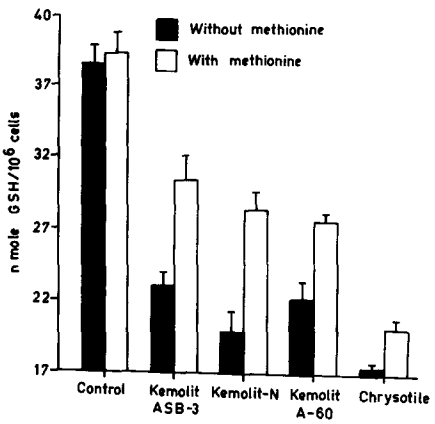

Figure 6. Effect of methionine on GSH content in control and dusts treated hepatocytes.

to wollastonites. This also indicates its most toxic nature and shows a clear cut assocition between LPO and GSH and is in agreement with Anundi et al. (1979). Earlier studies provide evidence for the existence of these changes in cellular biochemistry associate them with cellular injury (Hogberg and Kristoferson, 1977). Later studies suggest that GSH depletion can readily impair the cells defense against the oxidative damage which may ultimately lead to cell death (Moldeus and Quanguan,1987).

In addition to GSH, several endogenous antioxidants serve to protect the cell against oxidative damage (Sies and Cadenas, 1983). Lipophilic antioxidant, α -tocopherol, has been considered as a primary protective factor against an attack on cellular membranes. Results clearly show that LPO is protected by α -tocopherol.Figure 3 shows that observed values were near to the contorl as also reported with different silicate dust by Rahman and Casciano (1985). This indicates that α -tocopherol completely inhibited LPO while efflux of intracellular GSH was not checked by it completely (Figure 4) as also reported by Pascoe et al. (1987). It has been suggested that LPO does not proceed until α -tocopherol has been substantially depleted (Cadenas et al. 1984, Hill and Burk, 1984). It is believed that α-tocopherol inhibits LPO process by the donation of hydrogen atom to the lipid radicals and thereby terminating the propagative process (McCay, 1985). Although the mechanism of α -tocopherol mediated maintenance of intracellular GSH levels in this system is not clear, since none of the enzymes invovled in the biosynthetic process of GSH are membrane associated.

Figure 5 and 6 show a comparative values of LPO and GSH in presence and absence of methionine. Results indicate significant inhibition of MDA formation in treated hepatocytes suspension supplemented with methionine as compared to those lacking methionine. A slight decrease in the value of LPO in control cells was also observed, although it was not decreased to any significant degree (Figure 5). In a parallel study, an increase in intracellular GSH in methionine supplemented hepatocytes suspension was observed over those devoid of methionine (Figure 6). The results are in agreement with the work done by Annundi et al. (1979). This indicates active participation of methionin in maintaining the intracellular GSH content of hepatocytes. Since it has been suggested that it stimulates GSH synthesis in liver (Hogberg and Kristoferson, 1977). It can thus be summarized that our results strongly support the theory that GSH deficient isolated hepatocytes can undergo LPO rapidly enough to destroy the cells.

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