RAMAN SPECTROSCOPIC STUDIES ON THE MECHANISMS OF MEMBRANE DAMAGE INDUCED BY QUARTZ AND THE PROTECTIVE EFFECT OF ALUMINIUM CITRATE

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ABSTRACT

The molecular mechanisms of membrane damage induced by quartz and the antagonistic effect of aluminium citrate (Al citrate) against its toxicity were studied with liposomes by laser Raman spectroscopy. Liposomes under the action of quartz represented a Raman spectrum, in which the choline band shifts about 2-3 cm^{-1} toward the lower frequency and its intensity reduces, while its width increases; the intensity ratio of 1127 cm^{-1} to 1093 cm^{-1} bands decreases, so does the intensity ratio of 2883 cm^{-1} to 2847 cm^{-1} bands. These results show that quartz can react on phospholipids and form a stable complex with their polar head groups, $-N+(CH_3)_3$. The change of hydrocarbon chain conformation is caused by the interaction of quartz with the heads of phospholipids and deformation of liposomes. However, the similar change of choline groups cannot be found in Raman spectrum of liposomes under quartz pretreated with Al citrate or $AlCl_3$, and the order of hydrocarbon chain remains constant. The fact indicates further that Al citrate can exhibit its protective effect on membranes through the action of Al on the surface of quartz particles so that the direct interaction of quartz with lipidic molecules was blocked. As for titanium dioxide, it acts on liposomes too weak to cause any change of Raman spectrum, which is consistent with its weaker damage to the membranes.

INTRODUCTION

The injurious effect of quartz on cell membranes plays an important role in the pathogenic mechanism of silicosis. The significant effects of quartz on the functional and structural properties, such as fluidity, permeability, "water structure" and surface charge, of cell membranes as well as liposomes and the antagonistic effects of Al citrate were demonstrated in a series of our earlier experiments.[1-4] However, their molecular mechanisms remain to be clarified. For this reason, the studies of interactions of quartz, titanium dioxide and Al citrate with liposomes were carried out by laser Raman spectroscopy.

The use of Raman spectroscopy as a tool for research on the function and structure of membranes is a rapidly growing field. The quantitative molecular interpretations of the spectral events accompanying conformational alternations can be studied by this technique. Liposomes are often taken as a model for biomembranes because lipids in biomembranes are usually in a bilayer form. In the present study, we used dipalmitoyl-phosphatidylcholine (DPPC) to prepare liposomes due to its definite assignment of Raman bands.

In light of the finding that the injurious effect of quartz on membranes was lowered markedly by the pretreatment of these particles with Al citrate,[1-4] the present study was concentrated on the observations of Raman spectral effect of this pretreatment to elucidate its pharmacology.

MATERIALS AND METHODS

1. Quartz (99% pure) was supplied by Hygiene Institute of Chinese Prophylatic Medical Center. Titanium dioxide with a similar pure and size was obtained from Beijing Medical Chemical Factory. Al citrate with Al of 9.26% was supplied by Pharmaceutical Factory of Beijing Medical University. Quartz particles were pretreated with Al citrate or $AlCl_3$ using the method reported by Zou, T.T. et al.[5-6] DPPC was purchased from Sigma. Liposomes were prepared at the concentration of 100 mg/ml using the method described previously.

2. Raman spectra were obtained with Trimonochrometer Raman Spectrometer Model YJT-800. The 5145 A line of the laser was selected as excitation. The used power was 400-500 mw. Spectral slit width and scanning velocity were 800 μm and 1 cm^{-1}/sec, respectively. The computer was used to average signal, which was accumulated about 4-6 times. Spectra were not smoothed and only baseline was modified appropriately. Variance of sharp peaks were no more than 2 cm^{-1}. Qaulitative and quantitative analysis of the data were evaluated by the intensity ratio and order parameters to avoid interference.

RESULTS

C-N Band

715 cm^{-1} band in DPPC is the C-N stretching mode. The 1259 cm^{-1} bands are assigned to twisting and bending vibration modes of C-H, respectively, which are often utilized as an internal standard of C-N bands due to their high intensity and their insensitivity to environmental factors.

As shown in Table I and Figure 1, the 715 cm^{-1} band shifted about 2-3 cm^{-1} toward the lower frequency and its intensity was decreased markedly in DPPC + quartz group. As compared to DPPC control group, the intensity ratio of 715 cm^{-1} band to 1295 cm^{-1} or 1437 cm^{-1} band was reduced by 26.6% and 30.5% in 0.5 mg of quartz, respectively, while the reductions were more significant, that is 31.6% and 40.3%, respectively. Figure 2, the enlarged C-N band, illustrates clearly that the width of C-N peak was increased in DPPC + quartz group. However, these changes of C-N band caused by quartz almost disappeared by the pretreatment of quartz particles with Al citrate or $AlCl_3$. (Table I and Figures 1–2)

C-C Skeletal Structure

The skeletal optical modes in 1000-1200 cm^{-1} region include generally C-C and P-O stretching vibrations. Three principal bands to be used to estimate the intrachain order exist in this region. Figure 1 represents the Raman spectra in 1040-1150 cm^{-1} region of DPPC groups and its treated groups. The data listed in Table II show that quartz can decrease the intensity ratio of 1127 cm^{-1} to 1093 cm^{-1} bands and the intrachain parameter S_T, particularly in its 1.0 mg group, S_T was reduced by 21.6% compared to DPPC control. Whereas the pretreatment of Al citrate or $AlCl_3$ for quartz can partly reverse the effect of quartz, S_T tended to go uptowards. In contrast to quartz, these parameters did not change significantly in DPPC + titanium dioxide group, which means titanium dioxide cannot interfere with C-C skeletal structure of phospholipid membranes.

Table I

The Shift of C-N Band and Its Intensity Ratio to 1295 or 1437 Bands

Group	C-N cm^{-1}	I CN /I 1295 cm^{-1}		I CN /I 1437 cm^{-1}	
	$\bar{x}$	$\bar{x}$	%	$\bar{x}$	%
control	715.2	0.79	100.0	0.62	100.0
quartz (0.5mg)	713.2	0.58	73.4	0.43	69.5
(1.0mg)	712.4	0.54	68.4	0.37	59.7
quartz pretreated with Al citrate	715.2	0.79	100.0	0.57	91.9
quartz pretreated with $AlCl_3$	714.6	0.78	98.7	0.58	93.5
titanium dioxide	714.8	0.80	101.3	0.61	98.4

The doses of the pretreated quartz and titanium dioxide were 1.0 mg.

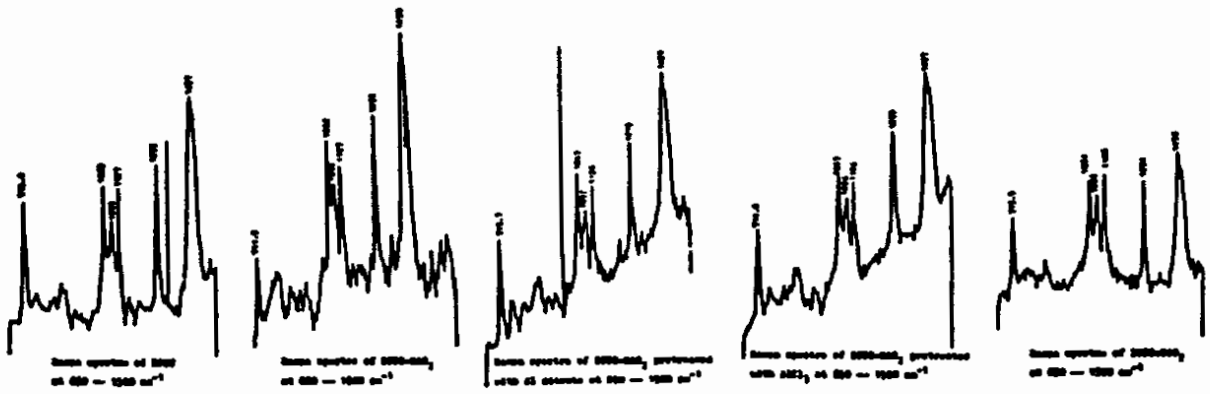

Figure 1.

C-H Stretching Vibration

The C-H stretching in 2800-2900 cm^{-1} region includes mainly the methylene symmetric stretch (2847 cm^{-1}) and asymmetric stretch (2882 cm^{-1}), which are used to evaluate the lateral packing order.

It can be seen from Table III and Figure 3 that the intensity ratio of 2882 cm^{-1} to 2847 cm^{-1} bands and the lateral order parameter S_L were lowered largely in two doses of quartz. Of interest, the intensity ratio and S_L values were enhanced markedly in both these pretreated groups, indicating that the interchain packing order was recovered partly. Likewise, there was no alternation in Raman spectrum in 2800-2900 cm^{-1} region of DPPC+titanium dioxide group. These findings suggest that titanium dioxide acted on liposomes weakly.

DISCUSSION

Raman spectra of lipids reflect mainly the vibrations of polar head group and C-H bonds. The 715 cm^{-1} band is the C-N symmetric stretching vibration mode. C-N group was even taken as an internal standard because it is constant and does not appear in the difference spectra.[7] It will be, therefore, a matter of great interest once the group has changed. The frequency of C-N band shifted, its intensity decreased and its width enlarged in DPPC under the action of quartz. This fact indicates that C-N group is an important site with which quartz interacts in membranes.

There a number of hydroxy groups on the surface of hydrated quartz, and silicic acid can be hydrolyzed partly to $HSiO_3^-$ in pH between neutral and alkaline due to its PK_1.[10-1] Whereas phospholipids of membranes rich in choline groups charged positively, which seems to provide $HSiO_3^-$ with a "target group," and a potential polar or ionic bond between N and O may be formed, resulting in their electrostatic and hydrophilic interactions.

The distinct changes of C-N band in Raman spectrum implicate that this binding is rather tight. According to this, it is explained why quartz can alter electrophoretic behaviour of macrophages and increase their negative charge density.[3] Also, the unhydrolyzed –OH groups of quartz surface form probably a hydrogen bond with $> P_2^-$ of phospholipid. On the other hand, deformation of liposomes to fit the globular surface of quartz particles may produce many binding sites

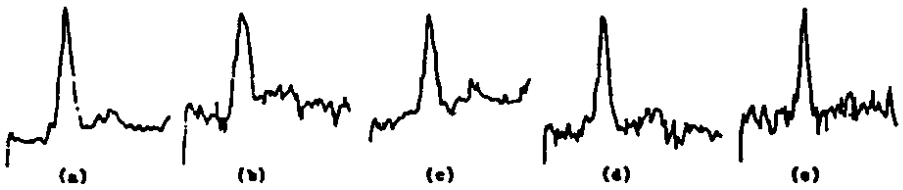

Figure 2. The enlarged C-N Raman band of DPPC (a), DPPC+SiO_2 (b), DPPC+SiO_2 pretreated with Al citrate (c), DPPC+SiO_2 pretreated with $AlCl_3$ (d), and DPPC+TiO_2(e).

Table II

The Intensity Ratio of 1127 to 1093 Bands and Order Parameter (S_T)

Groups	I 1127 cm^{-} /I 1093 cm^{-}	S_T	
	$\bar{X}$	$\bar{X}$	%
Control	1.36	0.74	100.0
quartz (0.5 mg)	1.24	0.68	91.9
(1.0 mg)	1.07	0.58	78.4
quartz pretreated with Al citrate	1.14	0.62	83.8
quartz pretreated with $AlCl_3$	1.17	0.64	86.5
titanium dioxide	1.39	0.76	102.7

The doses of the pretreated quartz and titanium dioxide were 1.0 mg.

Table III

The Intensity Ratio of 2882 to 2847 Band and Order Parameter (S_L)

Groups	I 2882 cm^{-1} /I 2847 cm^{-1}	S_L	
	$\bar{x}$	$\bar{x}$	%
Control	1.04	0.23	100.0
quartz (0.5 mg)	0.87	0.11	47.8
(1.0 mg)	0.90	0.13	56.5
quartz pretreated with Al citrate	1.02	0.21	91.3
quartz pretreated with $AlCl_3$	1.03	0.22	95.7
titanium dioxide	1.04	0.23	100.0

The doses of the pretreated quartz and titanium dioxide were 1.0 mg.

Figure 3. Raman spectra of DPPC (a), DPPC+SiO_2 (b), DPPC+SiO_2 pretreated with Al citrate (c), DPPC and SiO_2 pretreated with $AlCl_3$ (d), and DPPC+TiO_2 (e) at 2800–2900 cm^{-1}.

to force quartz and liposomes to form a tight complex. A good evidence for this is larger changes of bands at 2800-2900 cm^{-1} caused by quartz than that at 1040-1150 cm^{-1}.

The interaction of quartz with polar head group triggered a series of alternations of hydrocarbon chains. Membrane skeletal optical mode exists in 1000-1150 cm^{-1} region in Raman spectrum and is quite sensitive to the configuration of C-C bond. 1062 cm^1 and 1127 cm^{-1} bands are originated primarily from all-trans C-C stretching vibration, resulting in a zigzag chain on a plane, whereas 1093 cm^{-1} broad band is contributed by the Gauche vibration, which alters intrachain to a more disorder state. In general, information about the intrachain order and its molecular motion regulation may be obtained from the intensity rato of I1127 cm^{-1}/I1093 cm^{-1} and order parameter S_T.[7] The decreases in this intensity ratio and S_T resulting from the action of quartz on liposomes indicate a loss in the number of the trans isomers and an abnormal increase in the number of the Gauche isomers, leading the decreased intrachain order.[8]

The C-H vibration at high frequency region of 2800-2900 cm^{-1} is susceptible to the environmental factors and the direction of C-H chain. The intensity ratio of I2882 cm^{-1}/I2847 cm^{-1} and order parameter S_L are usually used to estimate the interchain order and its lateral molecular motion pattern.[7] The decrease of this intensity ratio and S_L induced by Quartz show that the lateral packing order was interfered simutaneously.

The molecular dynamic mechanism postulated in this paper was supported by the comparative studies of titanium dioxide and quartz pretreated with Al citrate or $AlCl_3$. It has been demonstrated that the affinity of titanium dioxide for quatenary ammonium group and Al^{3+} is much lower than quartz,[6-9] which is related to its hydration ability. Furthermore, titanic acid is so much weaker an acid than silicic acid that it is difficult to be hydrolyzed and to bind with C-N group, and also its ability to form hydrogen bond with $> P_2^-$ group is not as high as quartz. That is why titanium dioxide acts weakly on liposomes and no change of Raman spectrum could be found. It is more important that this is consistent with its weak cytotoxicity and its weak effects on cell membranes.

In light of the antagonistic effect of the pretreatment way against membrane damage by quartz, the present paper

centered on the observations of Raman spectroscopic effects of DPPC under the action of quartz particles pretreated with Al citrate or $AlCl_3$.

The results show that the changes of the main bands in the Raman spectra are very similar between the two pretreated groups. The frequency, intensity ratio and peak shape of C-N band were almost recovered to the control level. The intensity ratio of I2882 cm^{-1}/I2847 cm^{-1} and S_L went up markedly and closed to the control level. Whereas the intensity ratio of I1127 cm^{-1}/I1093 cm^{-1} and S_T were recovered only partly, indicating that the molecular degree of intrachain remained partly in the disorder. It seems therefore logical that Al citrate was unable to completely recover the effects of quartz on cell membranes, such as fluidity and permeability, to the control level. Moreover, it has been found that Al citrate did not alone affect Raman spectrum of DPPC in our preliminary experiment. So it is obvious that the effective component by which Al citrate exerts its phamacological effect is Al itself through the action on the surface of quartz particles.

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