THE EFFECT OF QUARTZ PARTICLES ON THE INCORPORATION OF C¹⁴-AMINO ACID AND C¹⁴-PALMITIC ACID INTO THE CELLS

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The effect of quartz particles on the incorporation of C¹⁴-leucine and C¹⁴-glycine into rat intraperitoneal macrophage protein was studied. The incorporations of C¹⁴-leucine and C¹⁴-glycine were inhibited very rapidly and remarkably by alkali-treated quartz particles, but not by ground quartz particles. And the incorporation of C¹⁴-palmitic acid into cell lipid was also strongly inhibited by alkali-treated quartz particles. The lactic acid production and the succinoxidase activity of the cells did not decrease by the addition of quartz particles under above experimental condition. So, it was assumed that the rapid decreases of incorporation of C¹⁴-leucine and C¹⁴-palmitic acid into the cell protein and lipid were the early sign of cell damage.

In 1963, the effect of the various silica particles on the oxygen uptake, glycolysis and C¹⁴-amino acid incorporation into cell protein of rat peritoneal macrophages was studied by Comolli¹⁾ and a marked inhibition of C¹⁴-leucine incorporation into the macrophage protein by tridymite particles was found. He pointed out that the first attack point of quartz particles to the biochemical process of the cells was protein synthesis. In the present paper, it was studied whether the incorporation of C¹⁴-amino acid into the cell protein was influenced by the quartz particles, whether the first attack point of quartz particles to the cells was the protein synthesis, and whether decreased rate of C¹⁴-amino acid incorporation into cell protein was produced by reduced adenosine triphosphate (ATP) content in the cells, since in the previous paper²⁾ it was found that the adenine nucleotide in the cells decreased very rapidly and remarkably by the addition of quartz particles. The incorporation of C¹⁴-palmitic acid into cell lipid was also studied.

MATERIALS AND METHODS

The Cells

The intraperitoneal macrophages of rats were collected by the method described in the previous paper.³⁾

Quartz

The two sorts of quartz particles with different surface structure were used for this experiment. One sample was ground quartz and the other was alkali-treated quartz. The preparing method and surface properties of these particles were described in the previous paper.³⁾ The sizes of both dusts were prepared to be from 0.5 to 2 μ and less than 0.5 μ by water sedimentation and centrifugation.

*The Estimation of Radioactivity**The incorporation of C^{14} -leucine or C^{14} -glycine into cell protein.*

In order to study the effect of the quartz particles on the protein synthesis system of the cells, the incorporation of glycine-1- C^{14} or DL-leucine-1- C^{14} into cell protein was observed. To the tubes containing 10 million macrophages suspended in Tyrode's solution 0.4 μ c of C^{14} -leucine or glycine was added and these tubes were incubated at 37°C for various periods of time. After incubation the incorporation was stopped with the addition of cold 10% TCA solution containing 0.1 M leucine or glycine. The precipitated protein was prepared for counting by the method of Rabinovitz *et al.*⁴⁾ The purified protein was dissolved in 88% formic acid, aliquots were added to planchets and plated as infinitely thin sample and then its radioactivity was measured with windowless gas flow counter.

The incorporation of palmitic acid into cell lipid.

Palmitic acid -1- C^{14} was dissolved in 0.05 N sodium hydroxide to give a stock solution. Prior to its use, this was diluted one in ten with 5% bovine albumin solution. This diluted labelled sodium palmitate-albumin solution was added to the cells. After incubation at 37°C for various periods of time, the medium was removed and the cells which were still adherent to the glass were washed twice with 10 ml of warm 0.9% sodium chloride. After centrifugation, these cells were again washed with 0.9% sodium chloride and re-centrifuged. The cell lipid was extracted with 10 ml of alcohol-ether 3:1 at 70°C for 1 hour and then this extract was evaporated to near dryness. Two milliliters of water was added and the lipid was extracted by 10 ml of 15% alcohol in petroleum ether (boiling point, 60 to 80°C). The aliquots of these extracts were added to planchets and counted in windowless gas flow counter.

RESULTS

Experimental 1. The inhibition of incorporation of C^{14} -leucine into cell protein of macrophages by quartz particles.

Five hundred micrograms of alkali-treated quartz particles less than 0.5 μ in diameter was added to 10 million cells and incubated at 37°C, and the inhibition of the rate of protein synthesis of the cells was observed. As

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seen in Fig. 1, in the control cells and the cells with ground quartz particles the incorporation of C^{14} -leucine into cell protein increased linearly for 3 hours, but in the cells added with alkali-treated quartz particles strong inhibition of the incorporation of C^{14} -amino acid was already observed after 1 hour-incubation. Further increase of uptake of C^{14} -amino acid was not observed after 2 and 3 hour-incubations.

Fig. 1. Effect of quartz particles on the C^{14} -leucine incorporation into cell protein. (quartz 500 $\mu\text{g}/1 \times 10^7$ cells)

The relationship between the dose of added alkali-treated quartz particles and the decrease of incorporation rate was linear as shown in Fig. 2.

Fig. 2. Relationship between the rate of incorporation of C^{14} -leucine and dust doses.

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The effect of size of alkali-treated quartz particles on the inhibition of C^{14} -leucine incorporation was shown in Fig. 3. The decrease of incorporation rate was more remarkable by particles below $0.5\ \mu$ than by those of 0.5 to $2\ \mu$.

Fig. 3. Effect of quartz particles on C^{14} -leucine incorporation into cell protein. (quartz 1mg / 2×10^7 cells)

The incorporation of C^{14} -glycine into cell protein was similar to that of C^{14} -leucine.

It was difficult to observe the early effect of quartz on the protein synthesis of the cells, since our counter did not work accurately for very low radioactivity. So, alkali-treated quartz particles below $0.5\ \mu$ in diameter were added to the cells incubated with C^{14} -leucine for 10 minutes, and the incorporation was determined at 3-, 5-, 10- and 30 minute-incubation after the addition of quartz. As seen in Fig. 4, the incorporation of C^{14} -leucine was already inhibited at 3 minute-incubation after addition of quartz particles as compared with control cells.

Experimental 2. The effect of quartz particles on the incorporation of C^{14} -leucine when adenine nucleotide or nucleoside was added.

In this experiment, C^{14} -leucine incorporation with the cells was studied using the cell-quartz mixture added with ATP or adenine or adenine and inosine in order to examine whether these nucleotide or nucleosides could mitigate the inhibitory effect of quartz on amino acid incorporation, since ATP in the cells regulates the rate of protein synthesis. These results are shown

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Fig. 4. Effect of quartz particles on the C^{14} -leucine incorporation at short-time incubation.

in Table 1. As seen in the Table, the addition of adenine or adenine and inosine or ATP did not recover the disturbed incorporation of C^{14} -leucine into cell protein induced by alkali-treated quartz particles. On the contrary, the addition of ATP or adenine and inosine reduced the incorporation of C^{14} -leucine into the control cells and especially ATP had remarkable effect.

Table 1. The effect of the addition of nucleotide or nucleoside.

Final concentration of nucleoside or nucleotide	Control (cpm)	Alkali-treated quartz (cpm)
(—)	8010	2267
$2 \times 10^{-2}M$ adenine	6878	2139
$2 \times 10^{-2}M$ adenine + $4 \times 10^{-3}M$ inosine	6856	2143
$5 \times 10^{-3}M$ ATP	3410	614

Experimental 3. The effect of quartz particles on the incorporation of C^{14} -palmitic acid.

The incorporation of C^{14} -palmitic acid into cell lipid was studied.

The rate of incorporation of C^{14} -palmitic acid into cell lipid was less than that of incorporation of C^{14} -amino acid into cell protein. To 30 million cells 1.5 mg of quartz particles less than 0.5μ in diameter was added and the mixtures were incubated with $0.4 \mu c$ of sodium palmitate-albumin solution for 1, 2 and 4 hours. In the case of the cells with ground quartz the uptake of

C^{14} -palmitic acid increased about 10% in comparison with that of controls. But, in the case of the cells with alkali-treated quartz particles, the incorporation into the cell lipid was inhibited about 70% after 1 hour-incubation. These results are shown in Fig. 5.

Fig. 5. Effect of quartz particles on the uptake of C^{14} -palmitic acid by macrophages. (quartz $1.5\text{mg}/3 \times 10^7$ cells)

Experimental 4. Comparative studies of the effect of quartz particles on the succinoxidase activity, lactic acid production, glucose consumption, the content of acid soluble nucleotide, TTC reducing capacity and C^{14} -leucine incorporation of macrophages.

Fig. 6. The comparison with various cellular functions by the addition of quartz particles.

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The effects of quartz particles of various doses on the above cellular functions were compared. These results are shown in Fig. 6. As seen in the Figure, the decrease of succinoxidase activity, lactic acid production and glucose consumption were more insensible than the incorporation rate of C^{14} -leucine into cell protein.

Experimental 5. The effect of quartz particles on the C^{14} -leucine labelled cells.

The decrease of incorporation of C^{14} -amino acid into cell protein was assumed to be due to the disturbed protein synthesis, but this drop might be produced by the increased release of amino acid from the cells. Therefore, the effect of quartz particles on C^{14} -leucine labelled cells was determined.

Before the addition of quartz particles 10 million cells was incubated in the medium containing $0.4 \mu\text{c}$ of C^{14} -leucine for 1 hour at 37°C and then $500 \mu\text{g}$ of alkali-treated quartz particles less than 0.5μ was added. After the incubation, the radioactivity of the cell-dust mixture separated for supernatant was determined. The effect of quartz particles on the labelled cell fraction was seen in Fig. 7. The radioactivity of the labelled cells with alkali-treated quartz particles decreased about 10% in comparison with that of the cells without dust. And radioactivity of the supernatant from the tube with alkali-treated quartz particles increased about 10% in comparison with that of supernatant of control.

Fig. 7. Effect of quartz particles on the C^{14} -labelled cells.

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From above results, it is clear that decrease of the uptake of labelled amino acid into the cells by quartz was produced by the disturbed protein synthesis.

This was also confirmed by the fact that C^{14} -leucine taken into the cells was found in 22,000 g supernatant of the macrophage homogenates.

DISCUSSION

In these experiments, it was demonstrated that C^{14} -amino acid of C^{14} -palmitic acid incorporation into the protein or lipid of the macrophages was inhibited remarkably by alkali-treated quartz particles and this inhibition appeared soon after the addition of quartz as seen in the case of acid phosphatase.³⁾

Role of adenine nucleotide was first assumed as the cause of this decreased amino acid incorporation by quartz, since protein synthesis depends on the adenine nucleotide and the amounts of adenine nucleotide in the cells were decreased by the quartz as reported in previous paper.²⁾ However, the addition of adenine nucleotides to the cell-quartz mixture could not restore the inhibited incorporation. Therefore, another mechanism should be considered for the decreased amino acid incorporation into the cells by quartz.

It seems very important that not only the amino acid or palmitic acid incorporation but also various functions of the macrophages were disturbed by quartz. As reported in this paper, succinoxidase activity, lactic acid production, glucose consumption and 2, 3, 5-triphenyl tetrazolium chloride (TTC) reducing capacity of the macrophages were decreased by the addition of quartz particles. Although some of them were inhibited very early and others not so early, these will suggest the damage of some cell part, or disturbance of some cell function which leads to a disorder of many cell functions as shown in this report.

It will be accepted very easily that cell membrane or phagosome membrane receives the damage or functional disturbance by quartz particles, but this first damage of cell membrane will be little, since labelled protein can not be released from labelled cells by quartz particles.

The following results many support above hypothesis, that is, within 3 minutes after the addition of quartz particles to the cells, many quartz particles were observed around cell membrane and phagocytosed particles were scarcely found in the cells by phase microscope.

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