

Dissolution of metals by human and rabbit alveolar macrophages

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ABSTRACT The ability of human and rabbit alveolar macrophages to dissolve 0.1–0.5 μm MnO_2 particles in vitro was compared. The amount of Mn added and dissolved from the particles over periods of nought, one, and three days was determined by flame atomic absorption spectrophotometry. The amount dissolved by human and rabbit macrophages was similar; on average 43.1% and 43.9%, respectively, were dissolved within three days. But rabbit and human macrophages dissolved significantly more Mn than was dissolved in the respective culture medium without macrophages after one and three days. It is suggested that the dissolution of particles by alveolar macrophages should be one basic component in any model of alveolar clearance of inorganic particles.

Recent experimental studies in man have shown that clearance of whole particles deposited in the alveolar part of the lung is a slow process; most of the particles clear with a half time of about one or several years.^{1–2a} The slow clearance of whole particles increases the importance of investigating the dissolution of particles in the lung. Even particles that are usually considered insoluble are dissolved in the lung.^{3–6} Although the alveolar macrophages phagocytose particles deposited in the alveoli, the ability of the macrophages to dissolve metal particles has not been measured quantitatively until recently.

Lundborg *et al* incubated samples of rabbit alveolar macrophages for nought to five days with MnO_2 particles and estimated the amount of Mn which then dissolved.⁷ The macrophages dissolved two to three times more Mn than the culture medium. The aim of the present study was to investigate whether human macrophages also have this ability to dissolve MnO_2 particles.

Material and methods

DESIGN

Human alveolar macrophages were lavaged from

eight volunteers. Alveolar macrophages were also lavaged from six apparently disease free male rabbits, weight 2–3 kg. On each day that human macrophages were lavaged (one or two subjects), macrophages were also lavaged from one rabbit.

The macrophages were incubated with MnO_2 particles, and the amount of Mn in soluble and in particulate form was measured after nought, one, and three days using conventional flame atomic absorption spectrophotometry (AAS). For each experiment with a sample of macrophages and MnO_2 particles a control experiment without macrophages was performed to study the dissolution of the MnO_2 particles in the medium.

HUMAN TEST SUBJECTS

Table 1 gives the personal data of the eight volunteers. Subject 1 underwent fiberoptic bronchoscopy because he had a left upper lobe infiltrate. After treatment with antibiotics, which started before the bronchoscopy, the infiltrate dissolved. In the other subjects the bronchoscopy was not performed because of clinical indications. All these subjects had normal chest x ray pictures. Subjects 2, 6, and 7 had a history of allergic rhinitis and subject 3 of asthmatic bronchitis, but none had any symptoms at the time of bronchoscopy. All eight subjects had normal bronchial mucosa except for a slight redness in some of the smokers.

Table 1 Personal and lavage data of volunteers

Patient No	Sex	Age	Smoking habits		FEV ₁ (% pred)	FVC (% pred)	Lavage volume (ml)	Recovered fluid (ml)	Total cell yield ($\times 10^6$)	Lymphocytes (%)
			Cig/day	Duration (years)						
1	M	62	0*	—	98	92	250	180	14.0	—
2	F	25	5	6	112	102	220	140	25.5	3.0
3	F	24	1-10	7	115	102	200	150	33.0	7.5
4	F	22	1-5	7	94	102	200	135	15.6	11.7
5	F	21	10-15	6	88	87	100	55	20.0	6.5
5	F	19	15	8	89	88	300	210	34.0	9.8
7	M	23	0	0	87	84	300	190	8.3	15.3
8	M	30	10	12	103	97	300	220	21.0	7.3

*Ceased five years ago.

MnO₂ PARTICLES

MnO₂ powder (Mallinckrodt, min 99.5% MnO₂) was suspended in deionised water. After 10 minutes of ultrasonic treatment the larger particles were allowed to sediment for 10 minutes. The water containing the smaller particles was then removed and centrifuged at 800 g for 10 minutes. The particles in this precipitate were then used for all the solubility experiments. Most of these particles were about one tenth or a few tenths of a μm , only 4% were larger than 0.4 μm .⁷ Before the particles were used they were heated for 30 minutes at 80°C.

MACROPHAGES

The human macrophages were lavaged from the right middle lobe using a modification of the method of Hoslam *et al.*⁸ A saline solution buffered to pH 7.0 with sodium bicarbonate was introduced and immediately gently aspirated again. The fluid was collected in a container kept on ice. The volume introduced ranged from 100 to 300 ml (table 1). The rabbit macrophages were lavaged with the same solution as that used for the human macrophages but otherwise were treated as described earlier.^{9,10}

The macrophage suspensions were centrifuged at 300 g for 10 minutes at room temperature and resuspended in 5 ml Hank's solution. The cell concentration was then estimated in a Bürker chamber. After a second centrifugation the cells were resuspended in Hank's solution and 0.4 ml samples were then placed on cover glasses and inserted into Leighton tubes. The cells were allowed to adhere for 20 minutes after which 2 ml of medium was added. The medium consisted of 15% serum and Hepes buffered Parker solution (pH 7.4) and contained 100 IU benzylpenicillin/ml and 100 μg streptomycin sulphate/ml. For the tubes with human macrophages, human serum (A B, Rh-Sabbatsberg's Hospital) was used and for the tubes with rabbit macrophages, rabbit serum (The National (Swedish) Bacteriological Laboratory). The Leighton tubes were placed in an incubator (ASSAB T 304 G) at

37°C, 5% CO₂ and 80% relative humidity for one hour. The medium was then decanted. From the number of cells added to the Leighton tubes and a count of the number of cells in the decanted medium the number of remaining cells was estimated to be $1.9 \pm 0.4 \times 10^6$ (mean \pm SD) for the human macrophages and $1.9 \pm 0.5 \times 10^6$ for the rabbit macrophages. Further 2 ml of fresh medium and the MnO₂ particles were then added to the tubes and the sample returned to the incubator. Parallel to each macrophage experiment a control experiment using the same medium and the same procedure but without macrophages was performed.

After nought, one, and three days the medium was removed and 1 ml 0.25% trypsin solution was added to each tube including the controls. After another 30 minutes in the incubator the tubes were placed in a vibrator for a few seconds. The contents of the Leighton tubes were then removed. Another 1 ml trypsin solution was added to the tubes and removed after 10 minutes. The removed contents of the tubes including 2 ml trypsin solution were mixed and centrifuged for about 10 minutes at 350 g. Half a millilitre of 1% Triton-X solution was added to the precipitate which was then treated ultrasonically for 40 minutes to disintegrate the cells. The precipitate and supernatant were then mixed and filtered through a 0.22 μm Millipore filter (type GS). The amount of Mn in the filter was taken to represent the amount of Mn in particulate form and the amount in the filtrate was taken to represent the amount in soluble form.

Mn ANALYSIS

All glassware and plastic tips were carefully washed with 10% nitric acid and rinsed several times with deionised water before use. Chemicals were of pro analysis quality (Merck). The filters were wet digested in glass beakers (50 ml) with 2 ml of a mixture of concentrated nitric acid and perchloric acid (HNO₃:HClO₄ 1:1) on a boiling waterbath for 20 minutes. Deionised water was added to an

Table 2 Human and alveolar macrophages were incubated with MnO_2 particles at 37°C for nought, one, and three days, whereafter the amounts of Mn added and dissolved were estimated. Parallel to each macrophage sample a control without macrophages was tested. (Data are expressed as mean \pm SD)

	0 days			1 day			3 days		
	Mn added (μ g)	Mn dissolved (μ g)	Mn dissolved (%)	Mn added (μ g)	Mn dissolved (μ g)	Mn dissolved (%)	Mn added (μ g)	Mn dissolved (μ g)	Mn dissolved (%)
Human macrophages (n = 8)	7.1 \pm 3.6	0.4 \pm 0.2	6.0 \pm 2.9	9.2 \pm 6.0	1.6 \pm 0.6	20.7 \pm 6.8	9.1 \pm 3.5	3.9 \pm 1.8	43.1 \pm 7.2
Human controls (n = 8)	10.7 \pm 6.5	0.4 \pm 0.2	4.5 \pm 1.5	11.2 \pm 7.3	1.2 \pm 0.7	11.9 \pm 4.7	11.5 \pm 4.9	2.6 \pm 0.9	25.3 \pm 7.8
Rabbit macrophages (n = 6)	7.3 \pm 3.9	0.3 \pm 0.2	4.9 \pm 1.9	7.9 \pm 3.6	1.6 \pm 0.7	22.8 \pm 9.5	9.1 \pm 4.1	4.0 \pm 2.0	43.9 \pm 9.3
Rabbit controls (n = 6)	9.1 \pm 4.6	0.3 \pm 0.2	3.9 \pm 2.7	7.5 \pm 4.0	0.5 \pm 0.3	6.9 \pm 2.5	9.0 \pm 5.2	1.6 \pm 1.0	16.9 \pm 5.0

appropriate amount (about 22 g). The filtrate was analysed without pretreatment against diluted standards. The manganese concentration in the solutions was determined by AAS, using a deuterium background correction (Perkin Elmer, model 403). All chemicals and materials used for the macrophage experiment, including the macrophages and the bronchoscopic equipment, were tested for manganese and were found to be in the order of the detection limit of the method or below. For details see Lundborg *et al.*⁷

STATISTICAL ANALYSIS

Paired *t* test was used to compare the macrophages and their controls and unpaired *t* test was used to compare human and rabbit samples without prediction of direction.

Results

Table 2 shows the amounts of Mn added to the human and rabbit macrophage samples and their controls. The dissolved Mn both in μ g and in percentage of the added amount is also shown. In the one day and three day samples the added amounts are similar for the four experimental conditions with the exception that the human controls received more Mn than the other three conditions. The human controls received significantly more Mn than the human macrophages in both the one day and three day samples ($p < 0.05$).

When dissolved Mn in the human macrophages

and human controls was compared the difference was statistically significant; for μ g dissolved the *p* values were < 0.01 and 0.02 for the one day and three day samples respectively, and for the percentage amount dissolved the corresponding *p* values were < 0.05 and < 0.002 . When the rabbit macrophages were compared with the rabbit controls both the μ g Mn and percentage Mn dissolved differed significantly in the one day and three day samples ($p < 0.001$).

When the human and rabbit controls were compared the μ g Mn dissolved differed significantly in the one day samples ($p < 0.05$) and almost significantly in the three day samples ($0.05 < p < 0.1$). The dissolved Mn in percentage differed significantly both in the one day and three day samples ($p < 0.05$).

Discussion

The average amount of Mn added in the different experimental conditions was close to 10 μ g but there were large variations. In our earlier study we studied the dissolution of Mn added within the range of a few μ g to about 100 μ g.⁷ Within this range the amount of Mn dissolved in μ g increased with the amount added, whereas the per cent Mn dissolved decreased with the amount Mn added. To investigate whether this was also the case in the present study the correlation coefficients for the four experimental conditions in the one day and three day samples were calculated (table 3). The indi-

Table 3 Correlation coefficient (*r*) between Mn added in μ g and Mn dissolved in μ g and per cent

	Dissolved Mn (μ g)		Dissolved Mn (%)	
	1 day	3 days	1 day	3 days
Human macrophages (n = 8)	+0.64	+0.89	-0.77	+0.04
Human controls (n = 8)	+0.59	+0.85	-0.53	-0.90
Rabbit macrophages (n = 6)	+0.86	+0.82	-0.73	-0.03
Rabbit controls (n = 6)	+0.63	+0.86	-0.59	-0.17

vidual *r*-values are uncertain owing to the small numbers of samples. Nevertheless, the general pattern of positive *r* values for dissolved Mn in μg and negative or very small positive *r* values for dissolved Mn in per cent agrees with the earlier results. This implies that in the present study the differences between macrophages and controls were not due to differences in the amount of added Mn as those differences were found in dissolved Mn in μg as well as dissolved Mn in per cent. The amount Mn dissolved by the rabbit macrophages and then controls agrees well with our earlier results.⁷ Human and rabbit macrophages dissolved MnO_2 particles to a similar degree but more was dissolved in the human than in the rabbit controls. The only difference between the control samples was that they consisted of human or rabbit serum.

Lundborg *et al* suggested that the reason larger amounts of Mn were dissolved in the macrophage samples than in the control samples was because of the low pH values in the lysosomes.⁷ In the lysosomes, pH values of 4–5 have been reported in the neutrophils and the peritoneal macrophages of rodents.^{11,12} This suggestion is supported by the fact that the amount of Mn dissolved in water at pH 6.5 was similar to the amount dissolved in the rabbit controls and that dissolution in water at pH 4.0 was complete within three days. The pH in the phagosomes of human neutrophils has been reported to be 6.0–6.5.¹³ We surmised that human alveolar macrophages might have a lower capacity to dissolve manganese than those in the rabbit macrophages, but this was apparently not the case.

If it is assumed that the dissolved Mn leaves the macrophages and the lung at a rate that is more rapid than the dissolution rate our *in vitro* tests indicate a clearance of MnO_2 particles from the lung with a half time of four days for both rabbits and man. This agrees fairly well with experiments in guinea pigs where the clearance of MnO_2 particles occurred with a half time of about two days after the first two days after inhalation.¹⁴ The somewhat faster clearance found in intact animals might be caused by some impairment of our *in vitro* macrophages to dissolve the particles. After three days, macrophages lost their ability to dissolve the MnO_2 particles more than the culture medium.⁷ Clearance of MnO_2 particles in man has been estimated to be 65 days.¹⁵ As rather small particles (mass mean diameter 0.9 μm) were used in this study, and as probably less MnO_2 per macrophage was given than in our *in vitro* study, the ability of the macrophages to dissolve Mn *in vitro* cannot explain the results. It

is not known whether this discrepancy can be explained by physicochemical differences between the particles, or by the binding of dissolved Mn to proteins in the human lung. As particles are phagocytised by macrophages, however, and as macrophages have the ability to dissolve metals, we believe that a model of alveolar clearance of particles from the lung must include the dissolution of the particles by alveolar macrophages.

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