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THE EFFECT OF CYSTEINE AND SODIUM SELENITE ON THE TOXICITY OF CADMIUM IN STAPHYLOCOCCUS AUREUS

By

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KORKEALA, H., A. UUSI-RAUVA and T. J. PEKKANEN: *The effect of cysteine and sodium selenite on the toxicity of cadmium in Staphylococcus aureus.* Acta vet. scand. 1981, *22*, 129—136. — Cysteine was shown to have a protective effect on the killing rate caused by cadmium (Cd) but no effect on the uptake of Cd in S. aureus 3719-. The addition of 254 $\times 10^{-3}$ mmol/l of sodium selenite or more into the liquid growth medium increased the lag phase of growth of S. aureus 3719-. Sodium selenite had no protective effect on the toxicity of Cd in S. aureus 3719- as evaluated by the length of the lag phase of growth in the presence of Cd and varying amounts of sodium selenite. 534 $\times$ 10^{-6} mmol/l of sodium selenite in the growth medium had no effect on the uptake of Cd in S. aureus.

cadmium uptake; lag phase; oxidative damage.

Tynecka et al. (1975) and *Gauthier & Flatau* (1977) have studied the effect of cysteine on the uptake of cadmium (Cd) in bacteria. *Tynecka et al.* observed that staphylococcal cells pretreated with cysteine were protected against the uptake of Cd. *Gauthier & Flatau,* on the other hand, found that the uptake of Cd in a marine bacterium belonging to genus Vibrio was increased by cysteine, which reduced the toxicity of Cd. The present study was undertaken to investigate the effect of cysteine on the toxicity of Cd for S. aureus, including the effect of the simultaneous addition of cysteine and Cd on the uptake of Cd by the bacterium.

Since it is known that selenium gives some protection against several of the toxic effects of Cd in experimental animals (e.g.

Parizek 1978), the effects of sodium selenite on the growth of S. aureus and on the toxicity of Cd in staphylococcal cells was also investigated.

MATERIAL AND METHODS

The test organism

The bacterial strain used in the study was Staphylococcus aureus 3719-*, which is sensitive to penicillin and Cd ions.

Chemicals and water

The $CdCl_2 \times 2½ H_2O$ (J. T. Baker, Phillipsburg, N.J., USA), $Na_2SeO_3 \times 5 H_2O$ and NaCl (E. Merck, Darmstadt, Federal Republic of Germany) were of pro analysis grade. L-cysteine was obtained from E. Merck and tris(hydroxymethyl)aminomethane from Sigma Chemical Co., St. Louis, Mo., USA. The carrier free $^{109}CdCl_2$ was a product of The Radiochemical Centre, Amersham, England (code CUS. 1). The water used throughout the experiments was double-distilled and deionized.

Effect of cysteine on the killing rate of S. aureus cells in isotonic saline containing Cd

Washed cells of S. aureus 3719- were incubated for 4 h at 35° C in a shaker with Cd or with Cd and cysteine together in an isotonic NaCl solution buffered to pH 8.0 with 0.2 mol/l tris/HCl buffer. The initial concentration of the bacterium in the media was always the same. A pH of 8.0 was chosen because most bacteria are generally more sensitive to Cd at high than at low pH (*Korkeala & Pekkanen* 1978). The Cd concentration used in the isotonic NaCl solution was 0.4 mmol/l and the L-cysteine concentration 0.8 mmol/l. The number of colony-forming units (CFU) was determined before and after 4 h of incubation on plate count agar (Difco Laboratories, Detroit, Mich., USA). Plate count agar plates were incubated for 48 h at 35° C. Six parallel experiments were made both with Cd and with Cd and cysteine combined.

Effect of cysteine on the toxicity of Cd in S. aureus, as determined by a disk assay technique

The medium used in the experiment was plate count agar. The pH of the medium was adjusted to pH 8 with 4 N-NaOH. Every petri dish contained 15 ml of substrate. The inoculum onto the petri dishes was taken from an S. aureus cell culture in the logarithmic phase of growth in nutrient broth (Orion Diagnostica, Espoo, Finland), containing 40×10^5 CFU/ml. On each petri dish were laid 2 filterpaper disks (Schleicher & Schüll, Dassel, Federal Republic of Germany,

* The strain was obtained from Dr. K. G. H. Dyke, Department of Biochemistry, University of Oxford, England.

Ø 12.7 mm): one containing 0.1 ml of Cd solution and the other containing 0.1 ml of Cd and cysteine solution. Ten plates were made using 0.01 mmol/l of Cd in one disk and 0.01 mmol/l of Cd plus 0.02 mmol/l of cysteine in the other, and another 10 using 0.02 mmol/l of Cd in one disk and 0.02 mmol/l of Cd plus 0.04 mmol/l of cysteine in the other. The plates were first kept for 2 h at 4° C and then incubated for 24 h at 35° C. After incubation the diameter of the inhibition zones of each disk was measured. The result is given as a mean of 2 perpendicular measurements.

Effect of cysteine and sodium selenite on the uptake of Cd in S. aureus

Staphylococcal cells from an overnight culture were exposed to Cd at 35° C in a shaker in isotonic NaCl solution. In the case of cysteine the cells were exposed for 4 h in isotonic NaCl solution containing 0.0001 mmol/l of Cd with radioactive $^{109}CdCl_2$ or 0.0001 mmol/l of Cd with radioactive $^{109}CdCl_2$ plus 0.0002 mmol/l of cysteine. In the case of sodium selenite the cells were exposed in isotonic NaCl solution containing 534×10^{-6} mmol/l of Cd with radioactive $^{109}CdCl_2$ or 534×10^{-6} mmol/l of Cd with radioactive $^{109}CdCl_2$ plus 534×10^{-6} mmol/l of sodium selenite. After exposure the cells were washed 4 times with distilled water and dried at 103° C; the radioactivity was then measured with an automatic gammasample counter (1280 Ultrogamma, Wallac Co., Turku, Finland).

Seven parallel experiments were carried out in the case of cysteine and 10 parallel experiments in the case of sodium selenite.

Effect of sodium selenite on the growth of S. aureus with and without Cd

The autoclaved yeast extract-glucose broth (denoted YG broth below) served as a basic medium. The broth contained 10 g of yeast extract (Difco) and 1 g of D-glucose (BDH Chemicals) per l distilled water. The pH of the broth was adjusted to 7.0. The filter-sterilized sodium selenite and Cd solutions were added to the broth immediately before inoculation, keeping the concentrations of the nutrients constant. Cells from an overnight culture were used for inoculation (0.1 ml of staphylococcal suspension to 7 ml of broth). The size of the inoculum was determined by plate count agar (Difco) and varied from 3×10^7 to 2×10^8 CFU/ml in different experiments. The tubes were incubated in a shaker at 35° C and growth was monitored with the Klett-Summerson photoelectric colorimeter (filter no. 42, Klett Manufacturing Co., N.Y., USA).

Initially the effect of sodium selenite on the growth of S. aureus was studied. In the first experiment the sodium selenite concentrations used were 0, 127×10^{-5} mmol/l, 127×10^{-4} mmol/l, 127×10^{-3} mmol/l, 1.27 mmol/l, and 12.7 mmol/l. No added Cd was used. Four parallel experiments for each concentration were performed. In the second experiment the sodium selenite concentrations used were 127×10^{-3} mmol/l, 254×10^{-3} mmol/l, 508×10^{-3} mmol/l and

1016×10^{-3} mmol/l. Again no added Cd was used. In this experiment 3 parallel experiments were performed.

In the third experiment the effect of sodium selenite on the toxicity of Cd was studied. The sodium selenite concentrations used were 267×10^{-6} mmol/l, 534×10^{-6} mmol/l and 1068×10^{-6} mmol/l. The Cd concentration was always the same, 534×10^{-6} mmol/l. Tubes without added sodium selenite or Cd and tubes with Cd (534×10^{-6} mmol/l) but without added sodium selenite served as controls. For each sodium selenite concentration with Cd, as also for the controls, 5 parallel tubes were incubated. Each such series was repeated 4 times.

RESULTS

In the experiments concerning the effect of cysteine on the recovery of Cd-stressed S. aureus cells, the initial mean logarithmic number of bacteria in the tubes was found to be 9.80 ± 0.07 (mean $\pm$ s). After 4 h of Cd treatment the corresponding figure was found to be 8.05 ± 0.05 (14 percent recovery) and after Cd and cysteine treatment 8.85 ± 0.03 (88 percent recovery). The difference between the mean logarithmic cell numbers of S. aureus before and after the 4 h Cd exposure was statistically significant ($P < 0.001$, Student's t-test) but the corresponding difference before and after Cd and cysteine treatment was not ($P > 0.05$).

In the experiments concerning the effect of cysteine on the toxicity of Cd by disk assay technique, the mean diameter ($\pm$ s) of the inhibition zone caused by Cd or Cd plus cysteine around the filter paper disks for 0.01 mmol/l of Cd was 18.6 ± 0.8 mm, for 0.01 mmol/l of Cd plus 0.02 mmol/l of cysteine 18.8 ± 0.4 mm, for 0.02 mmol/l of Cd 21.7 ± 0.7 mm, and for 0.02 mmol/l of Cd plus 0.04 mmol/l of cysteine 21.8 ± 0.5 mm. The diameter of the filter paper disks (12.7 mm) is included in the values of the inhibition zones.

The mean Cd content of S. aureus cells incubated for 4 h at 35° C with 0.0001 mmol/l of Cd in the shaker was 1.80 ± 0.27 µg/g of dried cells and the corresponding Cd content of staphylococcal cells incubated respectively with 0.0001 mmol/l of Cd and 0.0002 mmol/l of cysteine was 2.04 ± 0.27 µg/g of dried cells. The difference between the means was not significant ($P > 0.05$).

The effect of sodium selenite on the growth of S. aureus is shown in Table 1 and Fig. 1. When the sodium selenite concentrations of the YG broth increased over 254×10^{-3} mmol/l the lag phases of growth also increased.

Table 1. The effect of sodium selenite on the growth of Staphylococcus aureus 3719- in YG broth.

Concentration of sodium selenite in the YG broth	Klett reading[a]				
	Incubation period (hours)				
	0	2	4	6	8
0 mmol/l	0	7±4	66±14	153±3	191±6
127×10^{-5} mmol/l	0	7±5	64±8	149±3	188±12
127×10^{-4} mmol/l	0	6±2	65±7	159±7	198±8
127×10^{-3} mmol/l	0	5±2	46±6	136±6	204±7
1.27 mmol/l	0	2±1	4±2	5±1	6±1
12.7 mmol/l	0	2±1	6±4	5±4	5±4

[a] The growth of S. aureus 3719- in YG broth at 35° C with varying concentrations of sodium selenite was monitored by the Klett-Summerson photoelectric colorimeter (filter No. 42). Each value represents the mean ± s of 4 parallel experiments.

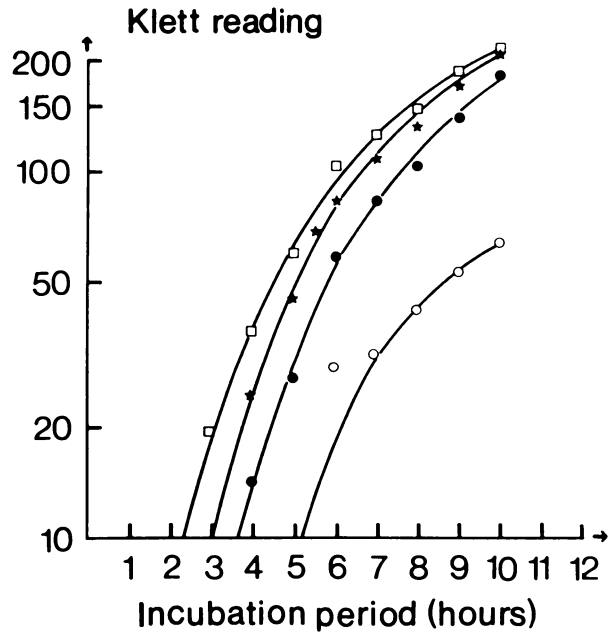

Figure 1. Growth of Staphylococcus aureus 3719- in YG broth with added filter-sterilized sodium selenite solution. Growth was monitored by the Klett-Summerson photoelectric colorimeter (filter No. 42). Each point represents the mean of 3 parallel experiments. Sodium selenite concentrations used were as follows (□) 127×10^{-3} mmol/l, (★) 254×10^{-3} mmol/l, (●) 508×10^{-3} mmol/l, and (○) 1016×10^{-3} mmol/l.

The effect of sodium selenite on the growth of S. aureus in YG broth containing 534×10^{-6} mmol/l of Cd is presented in Fig. 2. The figure gives the result of one experiment series. Parallel experiment series gave mostly similar results. Sodium selenite had no effect on the lag phase caused by Cd.

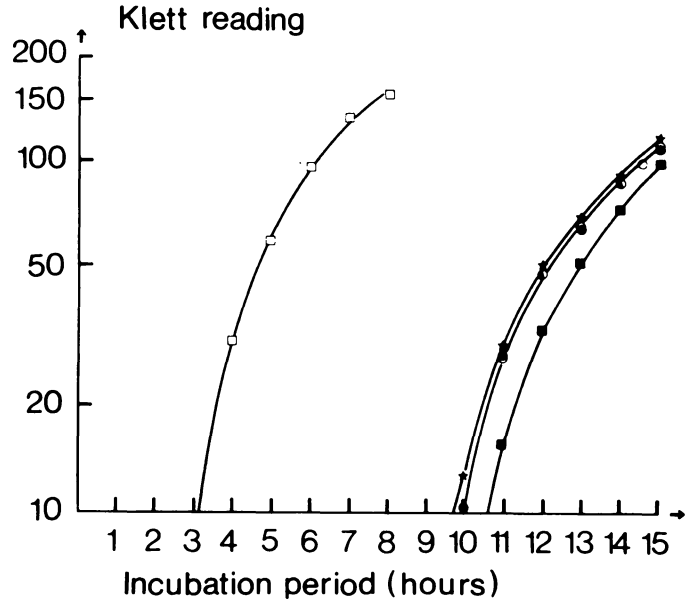

Figure 2. Growth of Staphylococcus aureus 3719- in YG broth with added filter-sterilized 534×10^{-6} mmol/l of Cd solution and 267×10^{-6}, 534×10^{-6} or 1068×10^{-6} mmol/l of filter-sterilized sodium selenite. Growth was monitored by Klett-Summerson photoelectric colorimeter (filter No. 42). Each point represents the mean of 5 parallel experiments. Symbols: (□) no added Cd or sodium selenite, (○) 534×10^{-6} mmol/l of Cd, (●) 534×10^{-6} mmol/l of Cd + 267×10^{-6} mmol/l of sodium selenite, (■) 534×10^{-6} mmol/l of Cd + 534×10^{-6} mmol/l of sodium selenite, and (★) 534×10^{-6} mmol/l of Cd + 1068×10^{-6} mmol/l of sodium selenite.

The mean Cd content of S. aureus cells incubated 2 h at 35° C with 534×10^{-6} mmol/l of Cd in the shaker was 1.01 ± 0.09 μg/g of dried cells and the corresponding mean in staphylococcal cells incubated respectively with 534×10^{-6} mmol/l of Cd and 534×10^{-6} mmol/l of sodium selenite was 1.05 ± 0.07 μg/g of dried cells. The difference between the means was not significant ($P > 0.05$).

DISCUSSION

The results showed that cysteine added simultaneously with Cd seems to reduce the killing rate of S. aureus caused by Cd. On the other hand, in the agar diffusion experiments using filter paper disks cysteine did not reduce the toxicity of Cd in S. aureus. Cysteine had no effect on the uptake of Cd in S. aureus.

Tynecka et al. (1975) found with S. aureus that pretreatment with cysteine decreased the uptake of Cd, but they did not add Cd and cysteine simultaneously to the broth as in the present studies. The action of cysteine to reduce the redox potential of the bacterial growth medium (*Willis* 1969) could well explain its protective effect in S. aureus as shown in the present experiments. Cysteine could protect the bacteria from the oxidative damage induced by Cd, as suggested by *Korkeala & Sankari* (1980) and *Korkeala* (1980). In the agar diffusion experiments, in which the bacteria were grown on the surface of the plates, the bacteria were exposed to atmospheric oxygen. This may explain the non-effect of cysteine on the toxicity of Cd.

The results which *Gauthier & Flatau* (1977) have obtained with a marine bacterium belonging to the genus Vibrio are not fully in accordance with the present results. According to them, cysteine increased the uptake but decreased the toxicity of Cd, whereas thioglycollate, a redox potential reducing agent, increased the toxic effects of Cd. Their results indicate a specific role for cysteine as a reducing agent with respect to Cd toxicity rather than a protective agent with respect to Cd-induced oxidative damage. However, the present study and the study of *Gauthier & Flatau* were carried out by differing methods and on different bacteria.

The addition of sodium selenite resulted in a longer lag phase in S. aureus in the broth containing 254×10^{-6} or more mmol/l of sodium selenite but not at lower concentrations (Table 1 and Fig. 1). The lower concentrations likewise had no beneficial effect on the growth of S. aureus (Table 1). Fig. 1 indicates that S. aureus can accommodate to the presence of toxic levels of sodium selenite.

In experimental animals selenium has a protective effect against the toxicity of Cd (e.g. *Parizek* 1978). In the present experiments with S. aureus sodium selenite did not shorten the lag phase of growth caused by Cd. It also had no effect on the uptake of Cd and thus apparently no protecting effect on the toxicity of Cd in S. aureus.

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SAMMANFATTNING

Effekten av cystein och natriumselenit på kadmiums toxiska inverkan på Staphylococcus aureus.

Resultaten visar att cystein hade en skyddande effekt mot den letala verkan av Cd men ingen inverkan på S. aureus cellernas upptagning av Cd. Tillsatsen av 254×10^{-3} mmol/l natriumselenit eller mera till det flytande näringssubstratet förlängde lagfasen hos S. aureus. Natriumselenit skyddade inte mot Cd:s toxicitet hos S. aureus 3719-. Detta evaluerades genom att mäta lagfasens längd när S. aureus 3719- inkuberades tillsammans med Cd och olika koncentrationer av natriumselenit. 534×10^{-6} mmol/l natriumselenit i näringssubstratet hade inte någon inverkan på upptagningen av Cd hos S. aureus.

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