Free Rad. Res. Comms., Vols. 12-13, pp. 479-488
Reprints available directly from the publisher
Photocopying permitted by license only

BACTERICIDAL ACTIVITY OF CATECHOLAMINE COPPER COMPLEXES

J. ARONOVITCH, D. GODINGER and G. CZAPSKI†

Department of Molecular Biology, School of Medicine and †Department of Physical Chemistry, The Hebrew University, Jerusalem 91010, Israel

Washed or growing *E. coli* cells are killed by epinephrine, norepinephrine or dopamine in the presence of non lethal concentrations of Cu(II). Killing is enhanced by anoxia and by sublethal concentrations of H_2O_2. The rate of killing is proportional to the rate of catecholamine oxidation. The copper epinephrine complex binds to *E. coli* cells, induces membrane damage and depletion of the cellular ATP pool. The cells may be partially protected by SOD or catalase but not by OH radical scavengers. Addition of H_2O_2 to cells which were sensitized by preincubation with the epinephrine-copper complex, causes rapid killing and DNA degradation. Sensitized cells are not protected by BSA.

KEY WORDS: Catecholamines, copper, bactericidal effect, ATP, membrane damage, DNA degradation.

INTRODUCTION

The antitumor activity and the cytotoxicity of catecholamines (CA), such as epinephrine (EN), dopamine (DA) or 6-hydroxydopamine (6-OHDA) for mammalian cells is mediated by oxygen derived radicals and by the reaction of quinone oxidation products with nucleophilic groups within the cell.[1-6] The rate of CA oxidation and the oxidizing species produced depend on the presence of transition metal ions, hydrogen peroxide and the reducing power of the cell.[7] Peroxides and oxyradicals generated by oxidation of CA have been implicated in the degeneration of CA neurons, and oxidation products of the analog 6-OHDA may damage both serotonine and CA neurons.[8]

CA oxidation reactions are usually mediated by transition metal ions. Seeking a simple model to study damaging CA oxidation reactions operating *in vivo* and taking into consideration that the concentration of free transition metal ions in biological systems under normal conditions is very low, we have investigated the bactericidal activity of μM concentrations of Cu(II) in presence of excess catecholamine, using *E. coli* cells as a model system for cells lacking CA specific transport systems.

MATERIALS AND METHODS

Materials

All reagents were of analytical grade. Reagents were dissolved in triple distilled water and used without further purification.

Potassium phosphate, sodium chloride, ammonium sulfate, magnesium sulfate, cupric sulfate and hydrogen peroxide (Merck); vitamin-free casamino acids, tryptone,

yeast extract and agar (Difco); 2[N-morpholino]ethanesulfonic acid (MES), superoxide dismutase (SOD), catalase, thiamine (B_1), adenosine 5′-triphosphate (ATP), firefly lantern extract, diethylenetriaminopentaacetic acid (DTPA) and catecholamines (CA) (Sigma); EDTA disodium salt and glucose (BDH); acetic acid (Frutarom); Tris (Serva).

Methods

Unless otherwise noted the concentrations of the reactants used were: $CuSO_4$ 4 μM, EN/Cu ratio 32:1, H_2O_2 0.5 mM, bacterial suspensions 4–6 $\times$ 10^7 cells/ml.

Preparation of CA-copper complexes. Chilled solutions of cupric sulfate and CA at pH 6.5 were mixed immediately before use.

Cultures. *E. coli* SR-9, *E. coli* K_{12} KL-16 and its recA mutant were grown in Davis minimal medium[9] supplemented with 1% casamino acids, 0.5% glucose and 1 μg/ml thiamine. All cultures were grown for at least three generations to late logarithmic phase (0.8 O.D. at 600 nm).

Preparation of cell suspensions. Cultures were sedimented by centrifugation washed twice in wash buffer (1 mM phosphate buffer pH 7.4 and 1 mM $MgSO_4$) and resuspended in reaction buffer (10 mM MES pH 6.5 and 1 mM $MgSO_4$) to 4–6 $\times$ 10^7 cells/ml.

Determination of viable counts (survival). Cell suspensions were incubated for 2 minutes at 37° and then the appropriate CA-copper complex and H_2O_2 were added in this order. Samples were withdrawn at various time intervals and diluted 1:10 in stop mixture (wash buffer supplemented with 0.1 mM DTPA or EDTA and 50 μg/ml catalase). After further serial dilutions in wash buffer the viable cell number (mean of 10 samples) was determined by a standard plate assay on L agar.

DNA. DNA was isolated from cells by the method of Marmur[10] modified to meet our experimental conditions. Electrophoretic studies were performed as previously described.[11]

ATP. Cellular ATP levels were estimated after extraction in boiling water for 2 minutes. ATP was measured by the firefly luciferase assay according to Stanley *et al.*[12]

RESULTS

CA form complexes with transition metal ions. Seeking a model system to study *in vivo* induction of damage by CA-copper and CA-iron complexes, we have treated washed log. phase, *E. coli* cells, with the complexes in the absence or in the presence of sublethal concentrations of H_2O_2, monitoring survival, DNA and membrane damage. Wild types cells were compared with mutants deficient in DNA repair or in adaptation of membrane fluidity to growth temperature. We have shown that the oxidation rate of EN at pH 6.5, in the presence of constant [Cu(II)] and [H_2O_2], depends on the EN/Cu(II) ratio.[13] Using 2–4 μM Cu(II) and an EN/Cu ratio of 32:1 at which about 60% of the copper is complexed at pH 6.5, the initial velocity of oxidation is about half of that obtained with an EN/Cu(II) ratio of 2:1 when about 20% of the copper is complexed. By varying the [EN] and using a constant, relatively non-toxic [Cu(II)] we could study the effects of EN oxidation rates on the inactivation rate of *E. coli* cells.

Incubation of washed 5×10^7/ml. *E. coli* cells in MES buffer pH 6.5 with EN (0.1–2 mM) or Cu(II) (4 μM) or H_2O_2 (1 mM) for 20 minutes at 37° had no effect on the viability of the cells. The 32:1 EN/Cu(II) mixture was considerably more toxic than free copper. Only 1% of the cells survived 30 minutes incubation with 2 μM complexed copper in the presence of excess EN. Upon addition of 0.5 mM H_2O_2, Cu(II) became slightly toxic, whereas the $(EN)_2$-Cu(II) complex induced rapid exponential killing at a rate of 50–90% per minute. Killing was accompanied by depolarization of the cytoplasmic membrane, efflux of potassium, depletion of the ATP pool of the cells and the appearance of DNA single and double strand breaks (ssb and dsb) in the extracted DNA.

When most of the copper was complexed in the form of $(EN)_2$-Cu(II) the survival curves were biphasic suggesting the involvement of two processes. Using constant concentrations of copper and H_2O_2 and increasing [EN] we found that the length of the first slow phase of killing, the "shoulder", increased with [EN], i.e. with the relative concentration of complexed copper and was inversely proportional to the initial rate of EN oxidation[13] (Figure 1). The killing rate constant of the fast phase decreased slightly with [EN]. The "shoulder" disappeared when the cells were preincubated for 5–10 minutes with the complex prior to the addition of H_2O_2. Preincubation with EN or with Cu had no effect.

Preincubation with the complex in presence of oxygen caused little killing (10–15%) but almost doubled the exponential killing rate constant upon addition of H_2O_2 (Table I) (Figure 2a). The incubation with the complex caused considerable membrane damage as demonstrated by depletion of the ATP pool of the cells (Figure 2b), leakage of potassium from the cells (data not presented) and an enhanced rate of DNA degradation upon addition of H_2O_2. The $(EN)_2$-Cu(II) complex was more toxic under anoxia causing 90% killing after 5 minutes in the absence of H_2O_2. The effects

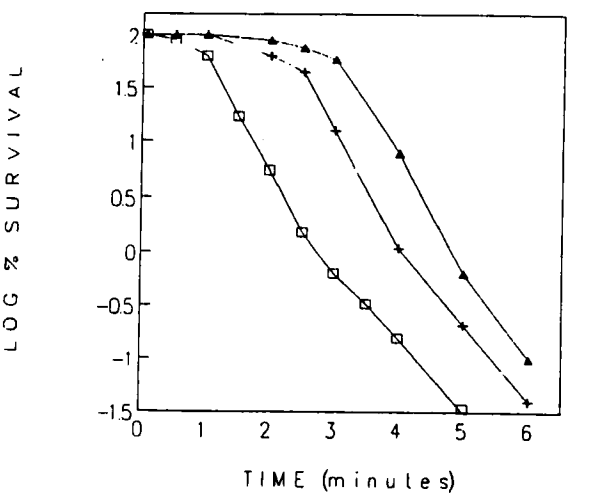

FIGURE 1 Effect of EN/Cu(II) ratio on the shape of the survival curves. Cu(II) 4 μM; variable [EN]; *E. coli* B cells 5×10^7/ml. ▲---▲ EN/Cu(II) ratio 32/1; +---+ EN/Cu(II) ratio 16/1; □---□ EN/Cu(II) ratio 2/1.

TABLE I

Effect of preincubation with $(EN)_2$-Cu(II), BSA, SOD or catalase on the ATP content and survival in the presence of H_2O_2.

Additives		ATP and survival at 0 min.		ATP and survival after add. of H_2O_2	
at time −10 min.	at time 0 min.	ATP % of control	percent survival	ATP % of control	K (min^{-1})
-	EN-Cu			6	0.63
-	EN-Cu + BSA			63	0.08
-	EN-Cu + SOD			12	0.075
EN-Cu	-	12	84	3	1.35
EN-Cu	BSA	11	80	7	0.64
EN-Cu + BSA	-	95	83	73	< 0.05
EN-Cu + SOD	-	40	80	8	0.85
EN-Cu + catalase	-	75	86	-	-

Cu(II) 4 μM; EN/Cu(II) ratio 32/1; H_2O_2 0.5 mM; BSA 200 μg/ml; SOD 50 μg/ml; catalase 4.5 μg/ml; MES buffer pH 6.5 10 mM; $MgSO_4$ 1 mM. *E. coli* B cells 5 × 10^7/ml were preincubated for 10 min with the complex and additives. H_2O_2 was added at time 0 min and the cells were incubated for additional 10 min. The ATP content of untreated cells was 40 nmoles per 10^8 cells. K, (min^{-1}) = exponential killing rate constant.

of incubation with $(EN)_2$-Cu(II) on washed or growing cells were not influenced by addition of OH· scavengers such as mannitol (0.05 M) or PEG (0.2%). ATP depletion was inhibited by BSA which prevents the binding of the complex to the cells without inhibiting EN oxidation. SOD and catalase partially protected against ATP depletion suggesting that both O_2^- and H_2O_2 produced by EN oxidation may be involved in the reactions induced by $(EN)_2$-Cu(II) oxidation. When BSA was added during preincubation with the complex or at time 0 together with the complex and H_2O_2, it inhibited both ATP depletion and loss of viability. However, when BSA was added

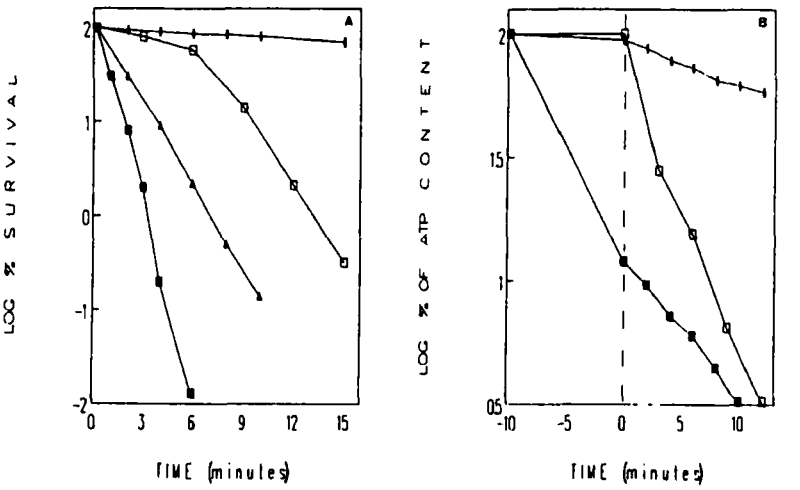

FIGURE 2 Effect of pretreatment with $(EN)_2$-Cu(II) on the survival and on the ATP pool of *E. coli* cells. Survival after pretreatment with the complex was 84%. H_2O_2 was added at time 0. A – Survival; B – ATP pool; □– – –□ without pretreatment with complex; ■– – –■ after pretreatment with complex; |– – –| BSA present during preincubation with the complex or added to the cells with the complex at time 0; ▲– – –▲ BSA added at time 0 to cells preincubated with the complex.

TABLE II
Effect of growth temperature and temperature shift on the killing rates by $(EN)_2$-Cu(II) and $(OP)_2$-Cu(II).

	Killing rate constant K, (min^{-1})			
Growth Temp:				
Preincubation	24°		42°	
Temp. (shift):	20°	42°	20°	42°
$(EN)_2$-Cu(II)	1.9	1.0	1.7	0.8
$(OP)_2$-Cu(II)	1.96			1.92

Preincubation: 10 min. in MES buffer pH 6.5, supplemented with 0.1% glucose.
Killing: Cu(II) 10 μM, EN/Cu ratio 2/1, H_2O_2 0.5 mM.
Cu(II) 2.5 μM, OP/Cu ratio 5/1, H_2O_2 0.5 mM.

after preincubation with the complex it did not prevent killing and only lowered the killing rate constant (Table I) suggesting that during the period of preincubation the complex binds strongly to cell components or becomes otherwise inaccessible to BSA.

Treatment of *E. coli* cells with $(EN)_2$-Cu(II) in the presence of sublethal concentrations of H_2O_2, without preincubation with the complex, also induces membrane damage. The transmembrane electrochemical gradient disappears and the potassium and ATP pools of the cells are depleted rapidly even in the presence of an energy source such as glucose (Figure 2b), while H_2O_2 or Cu(II) alone have only a small effect in the absence of EN. Additional lines of evidence support the assumption that the bacterial membrane is a target for the oxidative damage induced by EN oxidation. Cells grown at low temperature (24°) are more sensitive than cells grown at high temperature (42°). The latter have a lower concentration of unsaturated fatty acids in their membrane lipids and should therefore be less sensitive to lipid peroxidation. A short (5–10 minutes) preincubation of 42° grown cells at 20° sensitizes them to the level of cells grown at 24°. For comparison we used $(OP)_2$-Cu(II) which induces

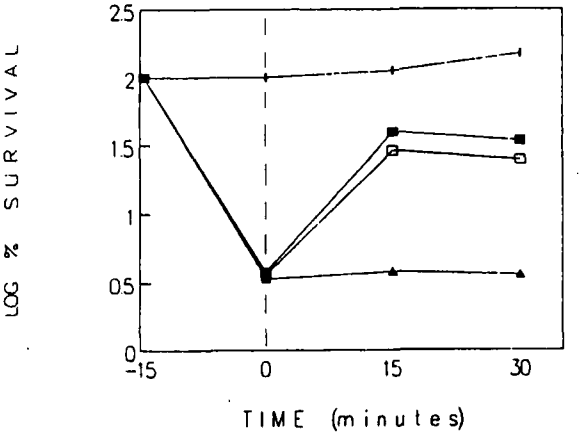

FIGURE 3 Survival repair after $(EN)_2$-Cu(II) treatment. Complex was added to *E. coli* B cells 5 × 10^7/ml. at −15 min. The reaction was stopped at 0 min. by the addition of 5 μM DTPA and the cultures were diluted 1:10 in prewarmed growth medium in the presence or in the absence of Chloramphenicol 100 μg/ml. |---| control of untreated cells incubated at 37°; ■---■ complex treated cells incubated at 37°; □---□ complex treated cells incubated at 37° + CM; ▲---▲ complex treated cells incubated at 5°.

mainly DNA degradation[14] and hardly affects the ATP pool. The survival after $(OP)_2$-Cu(II) treatment was not influenced by the growth temperature of the cells (Table II). Mutants deficient in the synthesis of unsaturated fatty acids (fab F1) were more resistant to EN than mutants which overproduce cis-vaccenic acid (fab F2). Both types of mutants are faulty in fluidity adaptation to temperature and both showed little change in sensitivity upon temperature shift (results not presented). About 90% of the cells "killed" by treatment with $(EN)_2$-Cu(II) without added H_2O_2 can be rescued by incubation under growth conditions if instead of diluting them in cold buffer after the reaction was stopped, they are diluted in warm growth medium and incubated for 5–15 minutes at 30–37°. A similar recovery of viability was observed with copper-ascorbate, which causes extensive membrane damage, but not with $(OP)_2$-Cu(II) treated cells.[15] Since DNA damaged cells behave differently, this observation also supports the assumption that the membrane is the main site of damage induced by EN. The membrane repair, the ATP replenishment and the recovery of viability are all energy and temperature dependent but do not require de novo protein synthesis (Figure 3).

The preincubation experiments suggested two possibilities, either the complex binds to or penetrates the cells slower than free copper ions and the shoulder represents the time required for the complex to reach critical targets in the cells. Alternatively, the "shoulder" may be due to the slower oxidation rate of the complex as compared to a mixture of complex, free Cu(II) and EN. Measuring the binding of the complex to *E. coli* cells either spectrophotometrically or by the use of radiolabeled EN, we found that the complex binds to the cells instantaneously, 10^8 cells bind approximately one nmole of the complex. The presence of cells reduces the initial oxidation rate of the complex but oxidation accelerates after a lag period of 5–10 minutes at 37°C. The sigmoidal oxidation curves obtained in the presence of cells resemble the oxidation curves of the DNA bound complex and probably reflect changes in the redox potential of the metal as a consequence of binding to the cells. Since a time dependent accumulation of EN was not observed, we preincubated the cells with $(EN)_2$-Cu(II) for various periods of time and then sedimented the cells, resuspended them in buffer and added H_2O_2. The length of the "shoulder" decreased and the killing rate increased with the length of the incubation period with the complex. The killing rate of cells incubated with the complex for 20 minutes was similar to the killing rate of cells incubated continuously in the presence of complex and H_2O_2 and depended only on the initial [$(EN)_2$-Cu(II)] (Figure 4). Considering that the complex binds to the cells instantaneously, the results indicate that a time dependent process which exposes sensitive sites in the cells, is required before ex-

TABLE III
Effect of incubation with $(EN)_2$-Cu(II) on the sensitivity of recA mutants.

Strain	Exponential killing rate constant K_1 (min^{-1})	
	Not incubated	Incubated
E. coli B SR-9	1.48	2.16
E. coli K_{12} KL16	1.53	2.88
E. coli K_{12} KL16 recA	0.83	5.30

Cu(II) 4 μM, EN/Cu(II) ratio 16/1, H_2O_2 1 mM. Cells were incubated with the complex for 7 minutes at 37°C prior to the addition of H_2O_2.

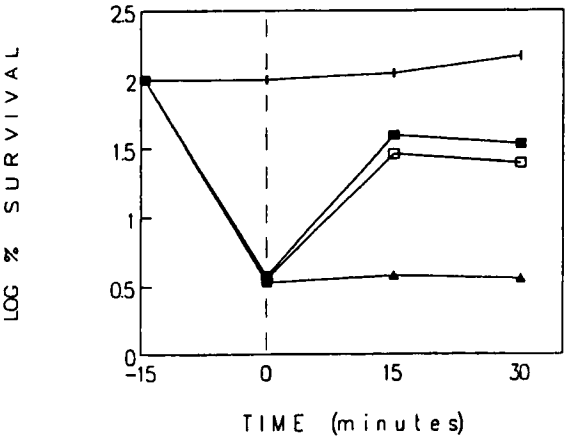

FIGURE 4 Effect of cell bound $(EN)_2$-Cu(II) on survival. *E. coli* B cells 5×10^7/ml were incubated for various periods of time with the complex, sedimented by centrifugation at 12.000 RPM for 5 min. at 20°, the supernatant was removed the cells were resuspended in buffer and incubated with H_2O_2 at 37°. Cells incubated with complex for: 1 min. □– – –□; 20 min. |– – –|; 40 min. ■– – –■; control, survival curve with complex added at time 0 ▲– – –▲ together with H_2O_2 and present continuously.

ponential killing may start. This assumption was tested by the use of recA mutants. When recA mutant cells were treated with the complex and H_2O_2 without preincubation, or with an EN/Cu(II) ratio of 4:1 which allows rapid oxidation of EN, washed recA mutant cells did not differ in sensitivity from the wild type parent cells. However, when most of the copper was complexed with EN and the recA cells were preincubated with the complex, the "shoulder" disappeared and the mutant became more sensitive than the parent strain (Table III). The enhanced rate of inactivation of recA cells after preincubation with the complex is probably due to DNA damage which presumably occurs only after the cell envelopes are damaged by preincubation with the complex. While cell death as a result of incubation with the complex in the absence of H_2O_2, or during rapid oxidation of EN in presence of H_2O_2 when the bulk of the Cu(II) is uncomplexed with EN, is probably due mainly to membrane damage. This conclusion is supported by the observation that DNA dsb are almost undetectable by electrophoresis on agarose (Figure 5), while membrane damage is evident and a high percentage of the "dead" cells can be rescued when they are incubated in the presence of an energy source.

Very little DNA damage (ssb) occurred during short periods (< 10 min.) of incubation with the complex. However, when hydrogen peroxide was added it induced rapid oxidation of EN, followed by additional membrane damage and DNA degradation. This was demonstrated in experiments which measured the extent of *in vivo* DNA damage after the addition of H_2O_2 as a function of the length of the preincubation period with the complex (Figure 5). These experiments demonstrate that *in vivo* DNA degradation and cleavage is proportional to the length of the preincubation period and suggest that membrane damage is a prerequisite for DNA damage. Similar results were obtained with copper complexes of norepinephrine and dopamine.

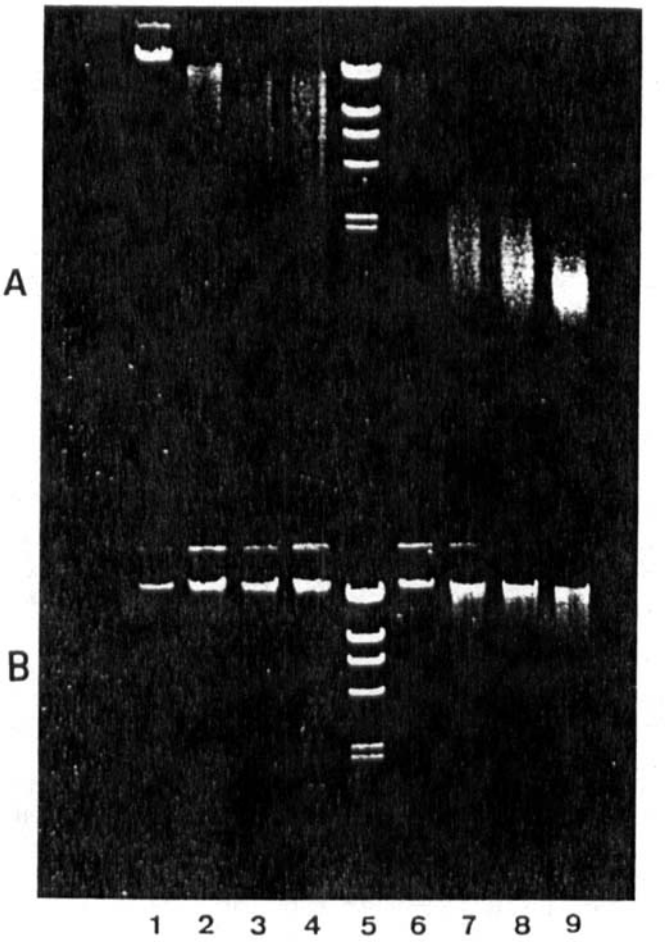

FIGURE 5 Electrophoretic profiles of DNA extracted from cells treated with $(EN)_2$-Cu(II) before the addition of H_2O_2. Cu(II) 8 μM; EN/Cu ratio 16/1. A – single strand breaks; B – double strand breaks; 1–4; after incubation with complex for 0, 10, 20, 40 min/ 5; Hind III digest, molecular weight marker. 6–9; 1–4 after additional incubation for 10 min with H_2O_2.

DISCUSSION

Epinephrine and dopamine are relatively not toxic to *E. coli* cells possibly because glucose grown cells lack specific transport systems for CA. When free metal ions are available, CA-metal complexes may be formed and these bind to the cell surface, induce oxidative membrane damage and cause cell death even in the absence of added H_2O_2. Anoxia markedly increases the toxicity of the complex as it enhances the toxicity of H_2O_2 for xthA mutants and the toxicity of $(OP)_2$-Cu(II) in the presence of ascorbate. It is not clear weather killing in the absence of oxygen involves redox cycling of the copper and if so, what is the source of reducing equivalents and what are the electron acceptors in the cells.[14,16–18]

Sublethal concentrations of H_2O_2 enhance killing and lead to DNA degradation. The rate of cell inactivation parallels the rate of EN oxidation. We therefore, assume that H_2O_2 creates a recycling redox system and as a result the complexes become very toxic, killing cells in micromolar concentrations.

We have shown that cell bound complex induces membrane damage which is partly repairable and that the fatty acid composition of membrane lipids determines the sensitivity of the cells. Similar effects were observed with near-ultraviolet and with γ-irradiated *E. coli* cells.[19-21] It is well established that lipid peroxidation modifies the molecular organization of biomembranes, changes the permeability of membranes and the activity of membranal enzymes such as ATP-ase. Similar changes were observed in cells treated with $(EN)_2$-Cu(II) in the presence or in the absence of added H_2O_2. We therefore assume that the EN-copper complex is oxidized on the surface of the cells, producing activated oxygen species which participate in membrane lipid oxidation reactions. This is supported by the observation that SOD and catalase protect the cells against the effects of cell bound complex and by the observation that oxidation of EN in the bulk of the reaction mixture, as in the presence of BSA, causes no cellular damage. The last observation and experiments with EN oxidation products such as adrenochrome support the assumption that the toxicity of the EN-copper complex is mainly mediated by oxygen derived radicals and the quinone oxidation products are relatively non toxic in the absence of copper ions and H_2O_2.

The primary target of EN induced killing seems to be the cytoplasmic membrane. The oxidation of cell bound $(EN)_2$-Cu(II) complex initiates membrane damage and enhances penetration of the complex into the cell. The slow phase of killing "shoulder" which follows the binding of the complex represents the time required to induce membrane damage which in turn exposes the cell content to the complex. Ternary complexes may then be formed with macromolecules such as DNA or protein. Oxidation of the bound complexes induces localized, site-specific reactions that damage the macromolecules, as demonstrated by the *in vivo* DNA cleavage observed.

The stability constant of the $(EN)_2$-Cu(II) complex at pH 6.8 is probably low since the addition of BSA prevents cell damage. However once the complex binds to the cell surface it induces membrane damage and becomes inaccesible to BSA. The presence of low concentrations of H_2O_2 forms a recycling redox system which induces DNA cleavage and degradation. These findings stress once more the biological significance of localized metal mediated reactions which occur on the surface of cells and macromolecules such as DNA and demonstrate that CA-copper complexes may induce oxidative cellular damage at physiological pH.

Acknowledgements

This study was conducted under the auspices of grants from the Council of Tobacco Research and the Israeli Academy of Science.

References

1. M.M. Wick (1982) Therapeutic effect of dopamine infusion on human malignant melanoma. *Cancer Treatment Reports*, **66**, 1657-1659.
2. P.S. Dasgupta and T. Lahiri (1987) Antitumor effect of i.p. dopamine in mice bearing Ehrlich ascites carcinoma. *Journal of Cancer Research and Clinical Oncology*, **113**, 363-368.
3. K. Nordlind and E. Sundstrom (1988) Different modulating effects of the monoamines adrenaline, noradrenaline and serotonine on the DNA synthesis response of human periphaeral blood T lym-

phocytes activated by mercuric chloride and nickel sulfate. *International Archives of Allergy and Applied Immunology*, **87**, 317–320.
4. D.B. McGregor, C.G. Riach, A. Brown, I. Edwards, D. Reynolds, K. West and S. Wellington (1988) Reactivity of catecholamines and related substances in the mouse lymphoma L5178y cell assay for mutagens. *Environmental and Molecular Mutagenesis*, **11**, 523–544.
5. G. Cohen and R.E. Heikkila (1974) The generation of hydrogen peroxide, superoxide radical and hydroxy radical by 6-hydroxydopamine, dialuric acid and related cytotoxic agents. *Journal of Biological Chemistry*, **249**, 2447–2452.
6. D.G. Graham, M. Tiffany, W.R. Bell Jr. and W.F. Gutknecht (1978) Autooxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine and related compounds toward C1300 neuroblastoma cells *in vitro*. *Molecular Pharmacology*, **14**, 644–653.
7. B. Bandy and A.J. Davison (1987) Interactions between metals, ligands and oxygen in the autooxidation of 6-hydroxydopamine: mechanisms by which metal chelation enhances inhibition by superoxide dismutase. *Archives of Biochemistry and Biophysics*, **259**, 305–315.
8. G. Cohen (1987) Oxygen radicals and Parkinson's disease. In *Oxygen Radicals and Tissue Injury* (ed. B. Halliwell), The Upjohn Company, pp. 130–135.
9. B.D. Davis and E.S. Mingioli (1950) Mutants of *E. coli* requiring methionine or vitamine B_{12}. *Journal of Bacteriology*, **60**, 17–28.
10. J. Marmur (1962) A procedure for the isolation of deoxyribonuc acid, from micro-organisms. *Journal of Molecular Biology*, **3**, 208–218.
11. J. Aronovitch, D. Godinger, A. Samuni and G. Czapski (1987) Ascorbid acid oxidation and DNA scission catalyzed by iron and copper chelates. *Free Radical Research Communications*, **2**, 241–258.
12. P.E. Stanley and S.G. Williams (1969) Use of the liquid scintillation spectrometer for determining adenosine triphosphate by the luciferase enzyme. *Analytical Biochemistry*, **29**, 381–392.
13. J. Aronovitch, D. Godinger and G. Czapski (1990) Hydrogen peroxide dependent oxidative degradation of DNA by copper-epinephrine. *Free Radical Research Communications*, this proceeding.
14. J. Aronovitch, A. Samuni, D. Godinger and G. Czapski (1986) *In vivo* degradation of bacterial DNA by H_2O_2 and O-phenanthroline. In *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine* (ed. G. Rotilio), Elsevier Science Publishers, pp. 346–348.
15. Unpublished results.
16. J. Aronovitch, A. Samuni, D. Godinger, M. Greenbaum and G. Czapski (1986) Bactericidal effect of H_2O_2 and DNA damage in xthA mutants of *E. coli*. in *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine* (ed. G. Rotilio), Elsevier Science Publishers, pp. 343–345.
17. J.A. Imlay and S. Linn (1986) Bimodal pattern of killing of DNA-repair-defective or anoxically grown *E. coli* by hydrogen peroxide. *Journal of Bacteriology*, **6**, 519–527.
18. J.A. Imlay and S. Linn (1988) DNA damage and oxygen radical toxicity. *Science*, **240**, 1302–1309.
19. M.B. Yatvin (1976) Evidence that survival of γ-irradiated *E. coli* is influenced by membrane fluidity. *International Journal of Radiation Biology*, **30**, 571–575.
20. M.B. Yatvin, J.J. Gipp and W.H. Dennis (1979) Influence of unsaturated fatty acids, membrane fluidity and oxygenation on the survival of an *E. coli* fatty acid auxotroph following γ-irradiation. *International Journal of Radiation Biology*, **35**, 539–548.
21. J.L. Redpath and L.K. Patterson (1978) The effect of membrane fatty acid composition on the radiosensitivity of *E. coli* K-1060. *Radiation Research*, **75**, 443–447.

Accepted by Prof. G. Czapski