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# EFFECT OF CARMINOMYCIN ON THE VIABILITY OF FIBROBLASTS FROM PATIENTS WITH REGULAR TRISOMY 21

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The sensitivity of three human fibroblast lines, trisomic with respect to chromosome 21, to an anthracycline antibiotic carminomycin was compared with that of a normal fibroblast line using a $^{51}Cr$ release assay. It was found that for an intermediate antibiotic concentration (10 $\mu$M) the sensitivity of trisomic fibroblasts, of increased content of Cu,Zn-superoxide dismutase was lower. These results suggest a role for superoxide-mediated membrane damage in the cytotoxic action of anthracycline antibiotics.

KEY WORDS: Carminomycin, fibroblasts, trisomy 21, Doun's syndrome.



## INTRODUCTION

Anthracycline antibiotics are commonly used in cancer therapy due to their cytotoxic properties. Two basic mechanisms have been proposed to account for the cytotoxic action of this group of antibiotics: (i) binding to DNA and interference with its biologic activity, and (ii) production of the superoxide radical anion and, indirectly, of other reactive oxygen species.[1,2] Anthracycline antibiotics are activated in cells to semiquinone forms by microsomal, nuclear envelope and plasma membrane electron transport chains.[3,4] Semiquinones of these antibiotics react with molecular oxygen yielding superoxide radical anion $O_2^{\cdot}$.[5,6] They may also form peroxide adducts of carbon-centered DNA radicals which decompose producing single and double breaks of the polynucleotide DNA strand.[7,8] A common outcome of these processes is the reduction of cell variability which may eventually lead to cell death.

Cells are known to possess an array of protective mechanisms preventing their damage by active oxygen species generated under physiological conditions or under the effects of external physico-chemical agents. They include enzymatic systems involving superoxide dismutase, (SOD), catalase and glutathione peroxidase, and low-molecular weight antioxidants. If the cytotoxicity of anthracyline antibiotics is mediated by reactive oxygen species, it can be expected that the effect of their action are different in cells differing in the level of antiradical/antioxidant protection systems.

Cells derived from human subjects with trisomy 21 may be seen as a natural test

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system for this question as they have a 50% higher activity of Cu,Zn-superoxide dismutase which is coded by a gene located in this chromosome.[9] This study was aimed at an investigation of the role of generation of the superoxide radical anion in the cytotoxic action of an anthracycline antibiotic, carminomycin, by comparison of its effects on fibroblasts with various levels of SOD activity, originating from normal and trisomic-21 donors.

## MATERIALS AND METHODS

### *Cells*

Human fibroblasts were derived from skin and fetal tissue from persons with Down's syndrome and from skin of a normal donor. They were obtained from tissue banks of the Center of Child Health, Warsaw, Poland and of the Institute of Medical Genetics of the Academy of Medical Sciences USSR, Moscow (Table 1).

### *Cell culture*

Cells were cultured by the classic methodology of diploid cell monolayers. Fibroblasts were grown in the Eagle Minimal Medium enriched with 10% calf serum and 15% lactalbumin hydrolysate (WSS, Lublin, Poland). Gentamycin was added at a concentration of 5–$\mu$g/ml medium. For the experiments, cells between the 5th and 15th passage were used. During the experiments, cells were incubated at a temperature of 37°C, in an atmosphere of 5% $CO_2$.

### *Measurement of SOD activity*

The activity of superoxide dismutase was determined in fibroblasts homogenates on the basis of inhibiton of NBT reduction in a xanthine-xanthine oxidase system.[10] Before the measurements the homogenates were centrifuged at 110000 × g in order to remove cell membranes.

### *Estimation of cell viability*

Fibroblasts in the stationary phase were labeled with $^{51}Cr$ for 4 hours by incubation in a medium containing radioactive chromium in the form of sodium chromate.

When studying the effect of carminomycin on the viability of fibroblasts, the $^{51}Cr$

TABLE 1
Cell lines studied

| Line | Source | Donor age | Supplier | Karyotype | Cu, Zn-SOD activity [u/mg protein] |
|---|---|---|---|---|---|
| T-74 | Tissue | Fetus | CCH,Warsaw | 47,XX,+21 | 16.9 ± 3.0[a] |
| T-107 | Tissue | Fetus | CCH,Warsaw | 47,XY,+21 | 15.5 ± 1.7 |
| B-1 | Skin | 10 months | IMG, Moscow | 47,XX,+21 | 17.1 ± 1.4 |
| B-2 | Skin | 7 months | IMG, Moscow | 46,XY | 11.4 ± 1.5 |

CCH - Center of Child Health, Warsaw, IMG - Institute of Medical Genetics of the Academy of Medical Sciences USSR, Moscow; [a]mean ± S.D., n = 4 or 5.

labeled cells were incubated in the minimal medium in the presence of the antibiotic for 3–42 hours. Then cell-free supernatant was removed and radioactivity released to the medium as well as the cell-associated radioactivity was measured in a Beckman LS 7800 scintillation counter. Each time point was measured in triplicate. The per cent of chromium released from the cells was assumed to represent the percentage of non-viable cells.

## RESULTS

Studies of the kinetics of $^{51}Cr$ release by human fibroblasts in the presence of various concentration of the anthracycline antibiotic carminomycin showed (Table 2) that at the lowest antibiotic concentration employed (1 $\mu$M) there were no significant differences in the response of control and trisomic cell lines to the drug, and the lower chromium release from trisomic cells may be attributed to the lower basal rate of release (in the absence of antibiotics). At the highest carminomycin concentration employed (100 $\mu$M) there was a massive cell damage so the results are difficult to interpret. However, the intermediate concentration used (10 $\mu$M) had a differential effect on normal and trisomic cells, the percentage of $^{51}Cr$ release being significantly

TABLE 2
Effect of carminomycin on the $^{51}$chromium release by various lines of human fibroblasts (mean ± SD; n = 5)

| Incubation time [hours] | Cell lines: Normal B2 | Trisomic B1 | Trisomic T74 | Trisomic T107 |
|---|---|---|---|---|
| | | % chromium release | | |
| | | No carminomycin | | |
| 3 | 14.1 ± 1.1 | ND | 9.0 ± 0.2 | ND |
| 6 | 14.9 ± 0.6 | 13.6 ± 0.5 | 11.4 ± 0.7 | 13.5 ± 1.1 |
| 18 | 24.1 ± 1.9 | 24.0 ± 0.7 | 18.7 ± 3.2 | 18.4 ± 6.0 |
| 30 | 36.2 ± 1.0 | 29.4 ± 0.3 | 28.5 ± 3.5 | 27.2 ± 0.2 |
| 42 | ND | 35.1 ± 1.7 | ND | 37.4 ± 0.4 |
| | | 1 $\mu$M carminomycin | | |
| 3 | 13.0 ± 1.0 | 10.2 ± 0.1 | 7.7 ± 0.8 | ND |
| 6 | 15.3 ± 0.9 | 16.1 ± 0.1 | 11.7 ± 2.5 | 13.7 ± 0.4 |
| 18 | 25.9 ± 1.6 | 23.5 ± 1.0 | 22.6 ± 4.9 | 21.0 ± 0.1 |
| 30 | 37.4 ± 2.1 | 29.5 ± 3.3 | 30.7 ± 0.7 | 30.0 ± 0.7 |
| 42 | ND | 36.2 ± 0.0 | ND | 37.0 ± 0.3 |
| | | 10 $\mu$M carminomycin | | |
| 3 | 12.4 ± 0.8 | 11.3 ± 0.2 | 8.9 ± 0.6 | ND |
| 6 | 17.8 ± 0.4 | 16.8 ± 0.7 | 14.3 ± 1.4 | 15.2 ± 0.1 |
| 18 | 44.6 ± 0.9 | 33.6 ± 3.3 | 36.4 ± 1.1 | |
| 30 | 65.6 ± 2.0 | 50.0 ± 2.8 | 52.1 ± 14.6 | 52.1 ± 1.0 |
| 42 | ND | 66.9 ± 0.7 | ND | 62.9 ± 1.1 |
| | | 100 $\mu$M carminomycin | | |
| 3 | 89.3 ± 4.5 | 79.1 ± 1.0 | 87.6 ± 7.3 | ND |
| 6 | 88.3 ± 4.6 | 83.2 ± 0.5 | 80.9 ± 1.7 | 83.0 ± 3.0 |
| 18 | 94.4 ± 3.8 | 78.2 ± 4.2 | 74.3 ± 5.1 | 80.1 ± 0.5 |
| 30 | 91.3 ± 1.6 | 81.4 ± 7.2 | 90.1 ± 6.4 | 80.2 ± 2.6 |
| 42 | ND | 75.7 ± 3.5 | ND | 79.5 ± 1.9 |

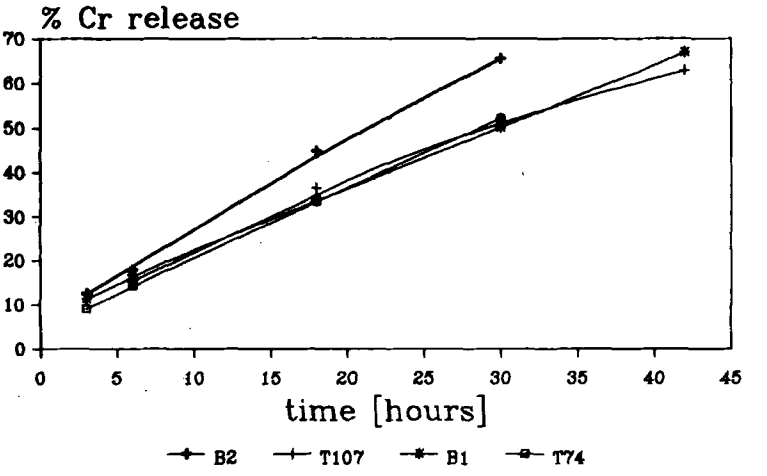


FIGURE 1 Effect of carminomycin (10 $\mu$M) on human fibroblasts: $^{51}Cr$ release by normal and trisomic human fibroblasts.

higher for the diploid fibroblast line B-2 than for the lines T-74, T-107 and B-1, triploid with respect to chromosome 21. A summary of results concerning the chromium release by the cells is shown in Figure 1.

## DISCUSSION

We have observed that fibroblast lines trisomic with respect to chromosome 21 and having therefore increased Cu,Zn-SOD activity are more resistant to carminomycin in a certain concentration range than diploid cells, of normal superoxide dismutase activity. These results suggest that the cell damage by the antibiotic measured in this experiment is mediated by the superoxide anion and/or reactive oxygen species produced of it.

The Cu, Zn—SOD is not the only antioxidant enzyme affected in fibroblasts trisomic with respect to chromosome 21. Mn-SOD was found to be decreased in these cells by an unknown effect[11] and increased glutathione peroxidase was reported[12] though not confirmed.[13] It seems to us, however, that the observed differences in the carminomycin sensitivity of trisomic fibroblast lines are most likely due to their increased content of Cu, Zn-SOD.

There is a controversy concerning the role of the reactive oxygen species in the cytotoxic action of anthracycline antibiotics. It has been observed that exogenous SOD, catalase and a compound of glutathione peroxidase-like activity (PZ51) protected MCF-7 human breast cancer cells to adriamycin cytotoxicity.[14] These results may also indicate that a part of the cytotoxic action of adriamycin is due to its effects on the plasma membrane as the protective enzymes were present in the extracellular medium. Sublines of MCF-7 cells resistant to adriamycin were found to have increased glutathione peroxidase activity[15] and in tumor cells, a relationship was observed between the cytotoxicity of anthracycline antibiotics and the $H_2O_2$ metabolism dependent on glutathione.[16]

On the other hand, no increased levels of antioxidant enzymes were detected in adriamycin-resistant lines of human lung cancer $GLC_4$ cells[17] and murine lymphoma P388 cells.[18] A recent comparison of adriamycine sensitivity of an oxygen-resistant subline of Chinese hamster ovary cells, of increased levels of glutathione, SOD, catalase and peroxidase did not differ from the parent line in adriamycin sensitivity estimated by clonogenic cell survival, after correction for differential drug uptake.[19]

Interpretation of these apparently divergent results is not easy at present. It can be suggested, however, that there is perhaps more than one target for the cytotoxic action of adriamycin antibiotics. Apart from their effects on DNA, also membrane damage may be important[20,21] and our results on induction of membrane permeability may differ from those obtained by clonogenic cell survival,[19] dependent rather on the DNA damage; membrane damage may be mediated to a higher extent by the superoxide radical anion and other reactive oxygen species than DNA damage.

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