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Effects of irradiation and THP-Adriamycin on the proteinase activity profiles in cultured V79 cells

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In the present work the changes of the activities of three types of proteinases (aspartic, cystein and neutral) in proliferative Chinese hamster lung fibroblasts (V79), treated by gamma irradiation or by the cytostatic agent 4-0-tetrahydropyranil (THP) Adriamycin, were followed. Our results show, that the activities of different enzymes tested, were changed by each of the two treatments in a different way.

Key words: fibroblasts; radiation effects; dexorubicin; peptide peptidohydrolases

Introduction

Proteolytic enzymes are essential in the cell metabolism and physiology, but they are also of crucial importance in processes involved in cellular proliferation kinetics and cell death.[1,2] They are an important part of gene regulation processes.[3] Their activity therefore, if measured when cells are in various physiological states or if they are exposed to damaging agents, may provide certain evidence about some molecular events occurring in the cells under particular circumstances.

In our previous papers we presented results indicating a correlation between the activity of intracellular proteinases and irradiation[4] and about proteinases influencing the repair of potentially lethal damage.[5,6]

In this work we present some changes of the activities of three types of proteinases (aspartic, cystein and neutral) in proliferating Chinese hamster lung fibroblasts (V79), treated by gamma irradiation or by the cytostatic agent 4-0-tetrahydropyranil (THP) Adriamycin. The results show pronounced changes in the activities of neutral and cystein proteinases following irradiation, and changes in the activity of acid proteinases which are different and related to the particular agent. They also suggest their involvment in repair processes.

Materials and methods

Cell cultures and experimental procedure

Chinese hamster lung fibroblasts (V79), were cultured as monolayers in Eagle's minimal es-

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sential medium, supplemented with 10% calf serum. Cell cultures were prepared by plating 10^6 cells per Petri dish of 10 cm in diameter, and after two days of exponential growth (doubling time 12 hours), before full confluency was reached, cells were either irradiated or treated by THP-Adriamycin. Following treatment, cell cultures were kept at 37 °C and samples taken after different time intervals and stored at –20 °C until proteinase activity assay.

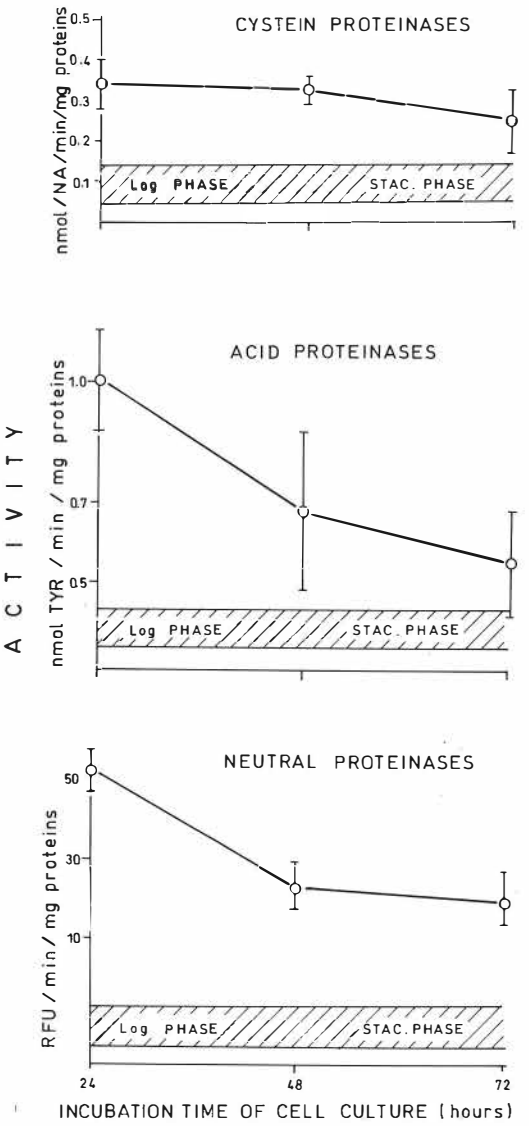

Figure 1. Changes of the proteinase activities in a growing culture of V79 cells. Activities of the cystein, acid and neutral proteinases are determined at different phases of cell growth and expressed per mg of proteins.

Proteinase activity

The activity of acid, neutral and cystein proteinases were determined by the following procedure: washed cells were harvested by a rubber policeman, concentrated by centrifugation, lysed in distilled water and frozen at –20 °C untill assay. Lysed cells were sonicated and homogenized by Pierce homogenizer. The proteinase activities in homogenates were determined using substrates: 2% bovine hemoglobine at pH 3.5 for acid proteinases. N-α-benzoyl-DL-arginine-1-naphthylamine (BANA) for cysteine proteinases and 1% calf thymus histones at pH 7.5 for the determination of neutral proteinase activity.[7,8]

Irradiation

For iradiation, a Gammacell 220 (Atomic Energy of Canada Ltd) unit was used. The dose rate was 4.13 Gy/min. Cells were irradiated at room temperature and then transferred to 37 °C until harvesting.

THP-Adriamycin treatment

THP-Adriamycin (4-0-tetrahydropyranil Adriamycin) was added to the growth medium to reach final concentration of 0.5 μg per ml. Cells were incubated in the Adriamycin-containing medium at 37 °C for one hour and then incubated until harvesting.

Results and discussion

In Figure 1 age-dependent changes of (a) cystein proteinases, (b) acid proteinases and (c) neutral proteinases in the growing culture are shown, and it is evident that in the phase of intensive cell growth acid and neutral proteinases decrease in their activity, while the cystein proteinases show a more steady-state pattern. When approaching the stationary phase, all

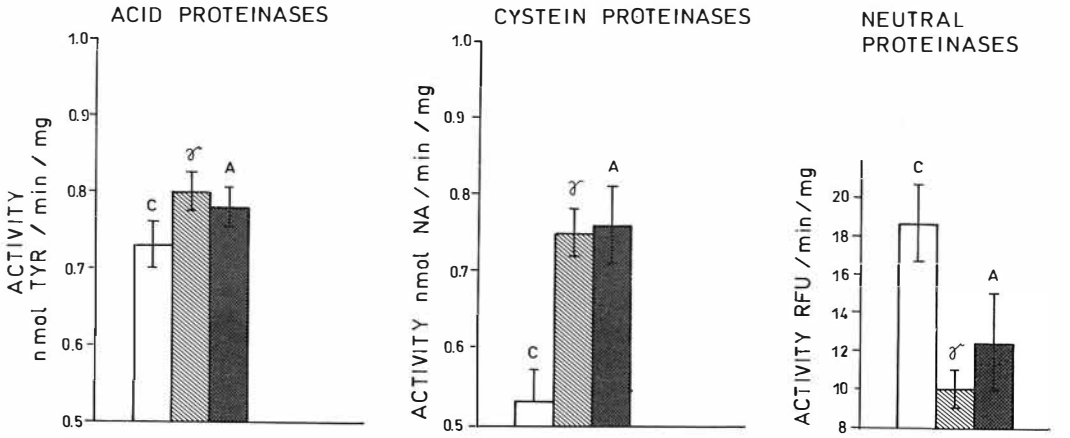

Figure 2. Proteinase activity profiles in cultures of V79 cells after gamma irradiation or treatment with THP-Adriamycin. C – Control values. γ – Cumulative data obtained by doses from 3-100Gy gamma rays 60 minutes following irradiation. A – Cumulative data obtained by 0.5 μg per ml THP-Adriamycin treatment for one hour. Proteinase activity was determined 60 minutes following the end of treatment.

three proteinases show a less intensive but similar decrease, and therefore this region of the culture age, with cells at the end of the exponential phase of growth was selected for treatment and proteinase activity assays. It was assumed that in this phase of cell growth the activities of all three proteinases have the most simillar patterns, thus being most convenient for comparison of the effects caused by various treatments.

Overall effects of gamma irradiation and of THP-Adriamycin on activity profiles of acid, neutral and cystein proteinases are presented in Figure 2, in order to show how treatment of each of those two agents will affect the activity of the proteinases in general. It is evident that

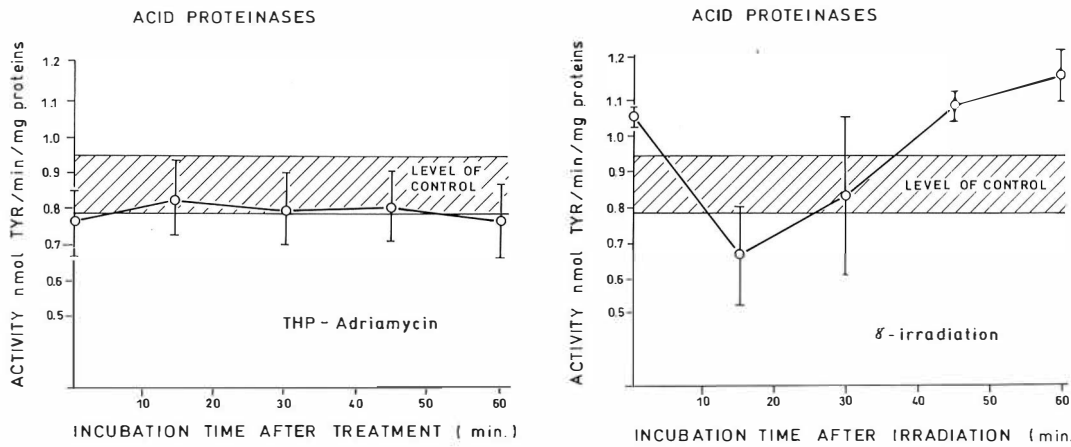

Figure 3. Time-dependent changes of acid proteinase activity cultures of V79 cells following treatment by THP-Adriamycin (a) or gamma irradiation (b). Cells were treated by THP-Adriamycin (0.5 μg per ml) for one hour, washed, and incubated in fresh medium. Dose of gamma irradiation was 15 Gy.

if assayed 60 minutes following treatment, acid proteinases are little affected, cystein proteinases reveal increased activity while neutral proteinases are signifficantly depressed if compared to their controls.

In Figure 3 we analysed the effects of gamma irradiation and of THP-Adriamycin on the acid proteinase activity during the first hour following treatment. The reason for selecting this particular proteinase was the apparent absence of effects of two agents on it. The two curves show clearly, however, that the patterns of time dependent effects are different. THP-Adriamycin did not influence the activity of the acid proteinase, irrespective of the time when the activity was measured during the one-hour period. The pattern of the effects obtain by irradiation however, shows a clear time dependence with a decrease at the 15th minute and an increase up to the 60 min. post treatment. Figure 4 shows that there is also dose-dependence of the activities of acid and cystein proteinases, if they are assayed one hour after irradiation with diferent doses of gamma rays.

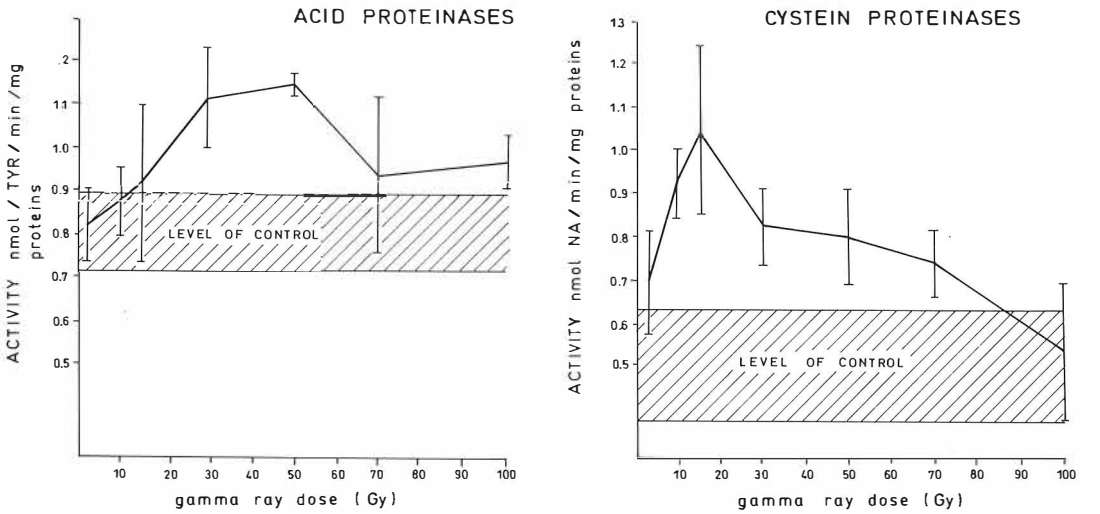

Figure 4. Dose-dependent changes of acid (a) and cystein (b) proteinase activity in cultures of V79 cells 60 minutes following gamma irradiation. Cells were irradiated, incubated for 60 minutes and then collected for proteinase activity assay.

Although they reveal different patterns, both proteinases have increased activities, significantly above the control levels, but with their maxima at different doses (50 and 15 Gy respectively), and with the tendency of decrease in the higher dose region.

The results show that the intensity of proteinase activity is dependent on the age of the cell culture, that THP-Adriamycin and gamma irradiation generally increase cystein proteinase activity and depress neutral proteinase activity, but have little influence on the acid proteinases. THP-Adriamycin has little influence on the acid proteinase activity for the first 60 minutes following treatment, but in opposite, gamma irradiation does change that activity, and finally, acid and cystein proteinase activities vary at different doses of irradiation, but in different patterns.

Genotoxic agents such as ionizing irradiation and cytostatics, commonly used in tumor therapy, damage and kill cells through different molecular mechanisms. Ionizing irradiation produces breaks in DNA molecules and this induces repair processes through activation of specific enzymes. Adriamycin, for instance as an

intercalating agent and so by distorting the DNA molecule inhibits the transcription of RNA (9). In that case there is no repair, at least not in that sense as after irradiation. Therefore, certain agent-related differences of the activities of intracellular proteinases were expected. According to the results presented in this work the activities of the three groups of intracellular proteinases responded in different ways to the treatments, and in brief, it can be concluded that they are an integral component of response of mammalian cells to irradiation and Adriamycin-produced damage. Which proteinases specifically are really envolved in these process, is the subject of furtner investigation.

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