INFLUENCE OF ENERGY METABOLISM AND MITOXANTRONE ON REPAIR PROCESSES FOLLOWING X-IRRADIATION IN CaNT TUMOURS

D Szeinfeld and S Wynchank

Research Institute for Medical Biophysics
Medical Research Council, Parow Valley, Cape, South Africa

Abstract

The response of the levels of adenosine-5'-triphosphate (ATP) and the specific activity of NADP-isocitrate dehydrogenase (NADP-ICDH) following 10 Gy x-irradiation in implanted CaNT tumours in the sternum of CBA mice is presented. Tumour ATP and NADP-ICDH levels appear to have crucial roles in repair processes after radiation damage. Mitoxantrone as an arresting drug is shown in this work to interfere with ATP yields in these tumours, hence it can modify cellular energy status and therefore be an effective way to modulate cellular repair processes after irradiation.

Introduction

Adenosine-5'-triphosphate appears to play a crucial role in repair mechanisms, including cellular repair following radiation damage. A very important and relevant repair process is that of DNA. The relationship of ATP to DNA repair has been studied by several workers, who have shown that some of the enzyme steps require ATP (1). Also, it has been suggested by some authors that ATP is associated with cell membrane repairs following ionizing radiation (2,3).

The oxidative reactions of the hexose monophosphate shunt and extramitochondrial NADP-isocitrate dehydrogenase reactions appear to be very important routes for generating NADPH, in addition to malic enzyme (NADP malate dehydrogenase) in the biosynthesis of fatty acids. These processes depend on the NADP/NADPH$_2$ ratio which is controlled in part by NADP-ICDH (4). There is presently a great deal of interest in combining ionizing radiation and anticancer drugs in the laboratory, with the aim of providing new strategies in the clinic. Neri et al (5) reported that mitoxantrone inhibited ATP production in rat heart slices. Thus this drug theoretically provides a means of inhibiting the increased energy production after irradiation, which may lead to enhanced tumour cell kill. Also mitoxantrone inhibits RNA and DNA synthesis (6, 7).

The purpose of this work is to investigate ATP content in experimental rodent tumours and the levels of NADP-ICDH related to energy status following x-irradiation solely, or after treatment with mitoxantrone as an arresting anticancer drug.

Materials and methods

Male CBA mice 2 to 3 months old were used. The tumour was maintained by several passage by innoculation of a tumour cell suspension subcutaneously into the sternum area of CBA mice. The CaNT tumour is a poorly differentiated adenocarcinoma and does not show detectable immunogenicity (8). The volume of tumour used in the investigation was between 150 and 250 mm^3. Mice were immobilized during irradiation by an acrylic restraining jig without anaesthesia

and were shielded with 3 mm thick lead, except for the tumour region. A Philips RT 100 x-ray set was used for the x-irradiations, (100 KVp, 8 mA), giving an HVL of 3mm Al and a dose rate of 7.36 Gy min^{-1} at the centre of the field and a total dose of 10 Gy. To prepare samples for intracellular ATP determinations, the tumour was cut away and immediately dropped into liquid N_2. Tumours were quickly weighed, then pulverized in a mortar with frequent additions of liquid N_2. One mℓ $HClO_4$ (6% w/v) was added, ground with the tissue, the mixture was allowed to become fluid and was homogeneized in a Potter-type glass homogeneizer. The homogenate was centrifuged at 17500g for 20 min at 4°C, the supernatant removed and the pH adjusted to 7.5 with 5M K_2CO_3. This was contrifuged at 17500g for 20 min and the supernatant was used for ATP determinations, according to the enzymatic method of Lamprecht and Trautschold (9).

Tumour samples for NADP-ICDH assay were prepared after sacrifice by cervical dislocation. The tumour was excised rapidly, tissue was blotted, weighed and homogeneized in a Potter-type glass homogenizer with ice-cold physiological saline 1:10 (w/v) which was 0.66 mM in EDTA. The homogenates were then centrifuged at 12000g for 20 min at 4°C and the supernatants were used for the NADP-ICDH assay, performed according to the method of Bernt and Bergmeyer (10). Protein content was determined according to the method of Lowry et al (11) using bovine serum albumin as a standard.

Mitoxantrone was administrated by intraperitoneal injection using a dose of 35 mg/m², 2.5h before the x-irradiation. Data were analyzed by using Student's 't' test.

Results

The levels of tumour ATP at different times after x-irradiation (10 Gy) show an augmentation compared with controls (3.42±0.47 nmol ATP/mg tissue, n = 36) which was first noted 45 min after irradiation (Fig 1). The maximum increases was observed 2.5h after receiving the x-rays (3.8 times that of the controls). Then 13h after irradiation, the ATP levels had returned almost to the control values.

After treatment with mitoxantrone in unirradiated tumours, the concentration of ATP was reduced by 0.76 times compared to controls ($P < 0.02$) and the levels of ATP following treatment with mitoxantrone and x-irradiation remained unchanged to within the experimental uncertainty ($P > 0.8$), compared to the values of tumour treatment with mitoxantrone only. The mean activity of NADP-ICDH was 0.46±0.07 µmoles isocitrate converted/min/mg protein as determined in 31 tumours. The activity of the enzyme increased following x-irradiation (10 Gy) to a maximum about 3h after irradiation (Fig 2).

Discussion

Adenosine-5'-trisphosphate has a major role in repair to intracellular damage after ionizing radiation and increased levels are most likely related to homeostatic regulation able to cope with physiological demands which follow radiation injury (12).

Sijens et al (3) have reported the response of a murine mammary carcinoma NU-82 in DBA-2 mice, to 10 Gy of gamma radiation. During the first 8h after this radiation the ratio ATP/inorganic phosphate increased. They hypothesise this effect is related to repair of radiation damage in particular to restoration of membranes. Benova et al (13, 14) reported that the protection of C57BL male mice from genetic radiation damage in germ-cell structures was achieved by using a combination of ATP, aminoethylisothiurorium Br-HBr and

serotonin. This reduced by half, the number of metaphases with translocations observed after 3 and 4 Gy x-rays to mouse spermatogonia, compared with irradiated mice not receiving the compounds. Removal of exogenous ATP from this combination led to a significant reduction (59%) in protective effect. NADP-ICDH mediated reactions are sources of reducing equivalents. The augmentation in the specific activity of this enzyme and its associated reducing equivalents may give protection against radiation damage. The enhanced activity of NADP linked dehydrogenase results from increased anabolic activity following radiation. Modification of the cellular energy supply by using cytotoxic compounds could therefore be an effective way to modulate cellular repair processes (15, 16). Mitoxantrone has been shown to decrease ATP production in rat heart slices in vitro (5) and here it is shown for the first time to decrease ATP production in tumours. Irrespective of the mechanism involved in the phenomena of repair, the present data show that if ATP levels fall as a result of interference from mitoxantrone, repair processes may be markedly inhibited as a result of reduced energy production. If the repair processes are slowed, a greater number of repairable lesions may be converted into lethal and non-repairable forms. Hence, for there to be effective repair processes, a higher ATP production must be superimposed upon the normal metabolic requirement for tumour cells.

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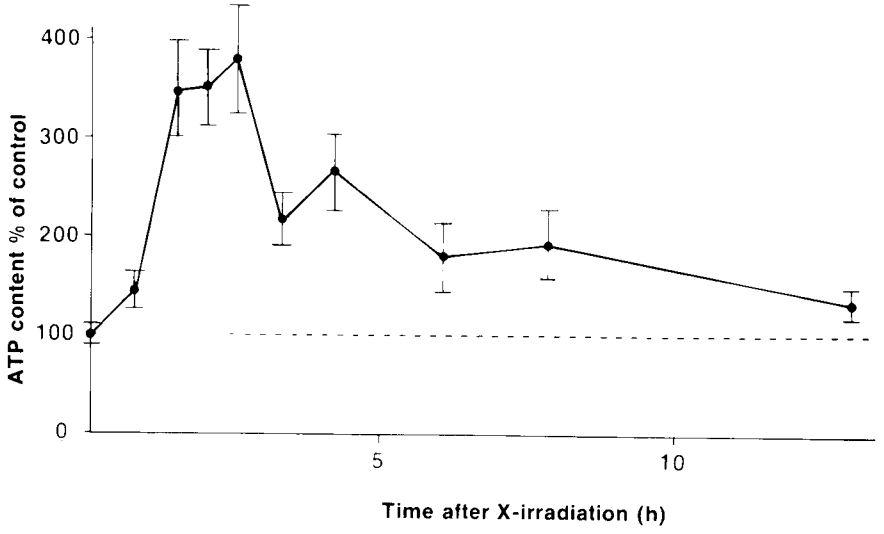

Fig 1 Radiation-related changes in ATP determinations from CaNT tumours in CBA mice at various times after irradiation. Data points represent the mean of at least three determinations ± SEM as indicated by the vertical bars. ATP levels after irradiation were significantly enhanced above levels in control tumours ($P < 0.05$).

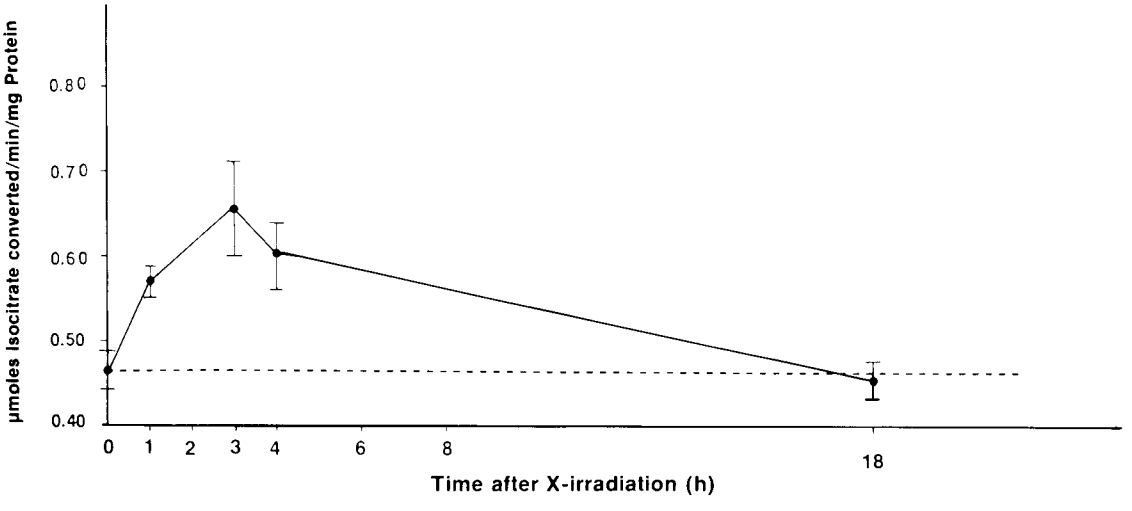

Fig 2 Radiation-related changes in NADP-Isocitrate dehydrogenase activity versus time in CaNT tumours in CBA mice. Each point represents the mean of not less than 5 values. SEM is indicated by vertical bars. This time variation was significantly different from the value obtained from unirradiated control tumours ($P < 0.05$).