Biochemical Radiosensitivity of Lymphoid Tumors

H. M. KLOUWEN* AND A. W. M. APPELMAN

WITH THE TECHNICAL ASSISTANCE OF C. M. J. ARTS

Radiobiological Institute TNO, Rijswijk Z.H., The Netherlands

Summary

The effect of whole-body irradiation on rat thymus, 3 different mouse lymphosarcomas, and a mouse myeloid leukemia were studied. In thymus, myeloid leukemia, and C57BL-lymphosarcoma, the inhibition of nuclear adenosine-5'-triphosphate (ATP) synthesis and mitochondrial oxidative phosphorylation is demonstrated within a few hours after irradiation. The inhibition of nuclear ATP synthesis precedes the decrease of weight of the tissues, and of the DNA and ATP content of the nuclei after irradiation. In the F1- and CBA-lymphosarcoma, inhibition of nuclear ATP synthesis is much more delayed and no inhibition of mitochondrial oxidative phosphorylation is observed at the time when nuclear ATP synthesis is inhibited 50%. The cells which show the early inhibition of nuclear and mitochondrial ATP synthesis died exclusively or predominantly in interphase while the more radioresistant lymphoma cells (F1 and CBA) died in reappearing mitosis.

An early biochemical mechanism for radiation-induced cell death is proposed.

Introduction

Investigations concerning early biochemical X-irradiation effects are mainly performed in radiosensitive organs such as thymus and spleen. In these tissues thymocytes and lymphocytes are the predominant cell type, and it is well known that within a few hours after whole-body irradiation with sublethal doses the majority of these cells show profound morphologic changes, e.g., pycnosis and karyorrhexis. It is therefore difficult to decide whether the biochemical changes which occur very soon after irradiation in these tissues are causally related to the cellular death and the mitotic inhibition.

A number of early biochemical radiation effects have been observed in thymus, e.g., inhibition of nuclear (10, 16) and mitochondrial oxidative phosphorylation (3), a loss of diphosphopyridine nucleotide (DPN) from thymocytes (20), inhibition of DNA synthesis (17), RNA metabolism (7, 15), depression of priming ability of DNA (12, 22), the release of histones (11) and cations (9), and a decrease of sulfhydryl groups (19) in the cell nucleus. Some of these radiation effects, e.g., loss of cations, the decrease of sulfhydryl groups and in particular the inhibition of nuclear adenosine-5'-triphosphate (ATP) synthesis, are demonstrable before visible cellular changes occur.

It is still insufficiently explored whether nuclear ATP synthesis is a general property of cell nuclei. Recently, we have investigated nuclear ATP synthesis in nuclei isolated from various types of cells (14). In this communication, results obtained with different mouse lymphosarcoma strains and a mouse myeloid leukemia will be presented in relation to results previously reported for thymocytes.

These results show that when a biochemical effect such as nuclear ATP synthesis is used as a parameter for radiosensitivity, variations in the response of the different cell types exist. Some tentative suggestions will be presented concerning a biochemical basis of radiation-induced cell death.

Materials and Methods

Male albino rats, weighing 130-150 gm, 6-8 weeks old, were used for the isolation of thymus nuclei (16). Transplantable tumors CBA/Ry., F1 (CBA x C57BL) lymphoma 30L, and a RF myeloid leukemia were used. Technical details concerning maintenance and passage of the tumors were described previously (21). Spleens of 7- to 10-week-old mice were generally used 7-12 days after injection of 10^5-10^6 tumor cells. At that time the spleens consisted predominantly of malignant cells and were depending on the tumor type 4-7 times the normal size. Nuclei from lymphosarcomatous and myeloid leukemia spleens were isolated according to the method described for the isolation of thymus nuclei.

Pieces of the tissues were fixed in Bouin's fluid and embedded in paraffin. Sections of 7 μ thickness were made and stained with hematoxylin and eosin.

Synthesis of ATP was determined after "aging" in vitro of the nuclei in 0.25 M sucrose-3 mM CaCl_2 at pH 7.0 under nitrogen for 15 min at 30°C and subsequent incubation in air for 30 min at 25°C. The difference between the ATP content of the nuclei kept under nitrogen and the ATP content after subsequent aerobic incubation represents the amount of ATP synthesized, as was described previously (4, 14). Acid-soluble extracts were isolated throughout from the nuclear sediments and ATP was determined using an enzymatic method (4). The acidified nuclear sediments were used for the determination of DNA (16). Mitochondrial oxidative phosphorylation was measured as described before (3).

X-irradiation of animals was performed at 50 cm F.T.D. with a dose rate of 68 rads/min (250 kv, 30 ma, HVL = 2.1 mm Cu).

Results

The effects of whole-body irradiation on nuclear ATP synthesis, the ATP and DNA content of the isolated nuclei, and on the...
weight of the organs are presented in Charts 1–6. In these charts, representative experiments are shown; variations or standard errors are not given in the figures. From the various results obtained it can be concluded that variations of the individual points are of the magnitude of ±10%.

It can be seen from these data that variations exist in the biochemical response of the cells after X-irradiation. In thymus, C57BL-lymphoma, and myeloid leukemia nuclear ATP synthesis is 50% inhibited between 2 and 3 hr after irradiation. Four hr after irradiation nuclear ATP synthesis has almost completely disappeared in rat thymus, while in the C57BL and myeloid leukemia the inhibition of nuclear ATP synthesis is less severe at that time. Previously, a slightly greater radiosensitivity of nuclear ATP synthesis in rat thymus has been reported (16), but apparently variations do occur under our experimental conditions.

In the CBA-lymphoma, a limited inhibition of nuclear ATP synthesis starts soon after irradiation and coincides with the radiation-induced changes in ATP and DNA contents of the isolated nuclei and in the weights of the organs. All these changes reflect apparently the destruction of cells. In the F1-lymphoma some inhibition (maximally 25%) of nuclear ATP synthesis is observed up to 12 hr post-irradiation, while during that period mitosis is completely arrested and the other biochemical parameters remain unaffected or even show a tendency to exceed the control values. Thereafter, inhibition of nuclear ATP synthesis progresses sharply and is followed closely by decreases in DNA and ATP contents and organ weights.

The results obtained with the CBA-lymphoma and F1-lymphoma cells show resemblance because the start of rapid and progressing inhibition of nuclear ATP synthesis coincides with the occurrence of cell death. On the other hand, in thymocytes, myeloid leukemia cells, and C57BL-lymphoma cells, the extent of radiation-induced inhibition of nuclear oxidative phosphorylation precedes the extent of visible cell death.

Especially the F1-lymphoma cells demonstrate a relative high radioresistance (Radiosensitivity and radioresistance are defined here in terms of biochemical and morphologic responses of cells after irradiation); therefore, much higher doses of radiation were then used in order to try to accelerate the occurrence of biochemical changes in these cells.

In some experiments the F1-lymphoma-bearing mice were irradiated with 10,000 rads. The results of these experiments are presented in Chart 5. It can be seen that an initial decrease of nuclear ATP synthesis occurs within the first 2 hr after irradiation. This decrease of nuclear ATP synthesis does not proceed as in the irradiated thymus, and there is even a tendency of repair of the biochemical damage. The radioresistance of the F1-lymphoma cells is impressive because up to 8 hr after irradiation with 10,000 rads mitoses are completely absent, while no pycnosis and cell death are observed.

The results presented in Table 1 demonstrate the considerable inhibition of nuclear ATP synthesis in thymocytes, myeloid leukemia cells, and C57BL-lymphoma cells within 2 hr after ir-
Biochemical Radiosensitivity of Lymphoid Tumors

Chart 3. Myeloid leukemic spleen (mouse), 900 rads. Control values (100%) are: spleen weight, 0.78 gm; DNA content per spleen, 9.1 mg; ATP content, 0.51 μmole/mg DNA-P; ATP synthesis, 0.34 μmole/mg DNA-P.

Chart 4. F1-lymphosarcomatous spleen (mouse), 900 rads. Control values (100%) are: spleen weight, 0.40 gm; DNA content per spleen, 4.1 mg; ATP content, 0.81 μmole/mg DNA-P; ATP synthesis, 0.55 μmole/mg DNA-P.

Chart 5. F1-lymphosarcomatous spleen (mouse), 10,000 rads. Control values (100%) are: spleen weight, 0.60 gm; DNA content per spleen, 5.0 mg; ATP content, 1.20 μmoles/mg DNA-P; ATP synthesis, 0.70 μmole/mg DNA-P.

Chart 6. CBA-lymphosarcomatous spleen (mouse), 900 rads. Control values (100%) are: spleen weight, 0.54 gm; DNA content per spleen, 7.1 mg; ATP content, 1.05 μmoles/mg DNA-P; ATP synthesis, 0.45 μmole/mg DNA-P.
radiation. In these cells the mitochondrial oxidative phosphorylation is inhibited 50%: +, 20-40% inhibition; —, no inhibition.

The effects of X-irradiation on mitochondrial oxidative phosphorylation are given at a point of time when nuclear oxidative phosphorylation is inhibited 50%: +, 20-40% inhibition; —, no inhibition.

The radiation-induced biochemical and morphologic responses of the cells studied are illustrated in Figure 1. In the myeloid leukemia cells death is then already apparent in many lymphoma cells. Interphase death or mitotic death. Interphase death is preceded by the occurrence of pycnosis which becomes visible between 1 and 2 hr after irradiation and occurs before the cells enter mitosis. On the other hand, mitotic death refers to that type of cell death which occurs in or immediately after mitosis after a period of mitotic delay. While interphase death is exclusively or predominantly seen in our experiments in thymocytes, myeloid leukemia cells, and C57BL-lymphoma cells, mitotic death is seen in the CBA-lymphoma and F1-lymphoma cells, respectively, after 4 hr and 12 hr post-irradiation.

It can be seen further from Table 1 that in the CBA- and F1-lymphoma cells used in our experiments no inhibition of mitochondrial oxidative phosphorylation is observed at a time when nuclear oxidative phosphorylation was inhibited 50%. Mitotic death is then already apparent in many lymphoma cells.

The time intervals after irradiation (900 rads) for 50% inhibition of nuclear oxidative phosphorylation are given in Table 1. Thymus and C57BL-lymphoma cells extensive pyenosis and karyorrhexis in thymus and lymphosarcomatous spleens.

It is therefore tempting to speculate that the damage observed near the nuclear membrane is the morphologic counterpart of inhibition of nuclear oxidative phosphorylation. Many of the radiation-induced biochemical changes mentioned in the introduction were not studied in relation with radiation-induced morphologic changes, and it is therefore not possible to consider any of these effects as inducers of the cellular radiation effects. 

When a causal relation exists between the biochemical and morphologic disturbances one would expect that these biochemical changes precede the occurrence of the morphologic changes.

It is difficult to evaluate such a supposed relationship quantitatively, and from the experiments presented in this paper it is only possible to conclude that in thymocytes, myeloid leukemia cells, and C57BL-lymphoma cells the extent of the radiation-induced biochemical disturbances, namely the inhibition of nuclear and mitochondrial oxidative phosphorylation, precedes the extent of recognizable damage (as observed after irradiation in thymus and lymphosarcomatous spleens).

This seems to us a suggestive reason to consider the inhibition of nuclear oxidative phosphorylation in combination with inhibition of mitochondrial oxidative phosphorylation as the principal biochemical lesions known so far associated with interphase death.

The other type of cell death—mitotic death—is apparently a direct result of damage of nucleoproteins because in the F1- and

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SUMMARY OF OBSERVED EFFECTS OF X-IRRADIATION ON LYMPHOID CELLS</strong></td>
</tr>
<tr>
<td>Time (hr) after irradiation (900 rads) of 50% inhibition of nuclear oxidative phosphorylation</td>
</tr>
<tr>
<td>Thymus</td>
</tr>
<tr>
<td>C57BL-lymphoma</td>
</tr>
<tr>
<td>Myeloid leukemia</td>
</tr>
<tr>
<td>CBA-lymphoma</td>
</tr>
<tr>
<td>F1-lymphoma</td>
</tr>
</tbody>
</table>

Two types of cell death are distinguished: M, mitotic death; I, interphase death. The C57BL-lymphoma cells die predominantly in interphase.

The effects of X-irradiation on mitochondrial oxidative phosphorylation are given at a point of time when nuclear oxidative phosphorylation is inhibited 50%: +, 20-40% inhibition; —, no inhibition.

Discussion

Using biochemical criteria, important variations are observed in the radiosensitivities of a number of lymphoma cells and a leukemia cell which are available in our laboratory. Generalizations concerning the type of tumor cells and their responses to radiation cannot be made, and the conclusions or hypotheses to be presented here are logically restricted to these particular tumor cells and thymic lymphocytes. The radiation-induced biochemical changes were studied in a 24-hr period after irradiation, while in the radioresistant F1-lymphoma cell the effects were followed up to 48 hr post-irradiation. Originally, nuclear ATP synthesis was considered to be a very radiosensitive process (10). More is known now about the mechanism of this process, which appeared to be an oxidative phosphorylation of the cell nucleus (5). In our laboratory different lymphatic and leukemic tumor strains were available and nuclear oxidative phosphorylation and the response of this process to X-irradiation could therefore be studied in a number of closely related cells. The results presented in this paper clearly demonstrate that nuclear oxidative phosphorylation can no longer be considered as a radiosensitive process per se, but that important variations exist in the radiation-induced biochemical and morphologic responses.

Recent biochemical results have provided evidence for the location of nuclear oxidative phosphorylation in or near the nuclear membrane (6). Braun observed that radiation-induced vacuolization near the nuclear membrane was the first sign of morphologic damage in thymic lymphocytes, whereas in thymic reticulum cells this vacuolization repaired (8). It is therefore tempting to speculate that the damage observed near the nuclear membrane is the morphologic counterpart of inhibition of nuclear oxidative phosphorylation. Many of the radiation-induced biochemical changes mentioned in the introduction were not studied in relation with radiation-induced morphologic changes, and it is therefore not possible to consider any of these effects as inducers of the cellular radiation effects.

When a causal relation exists between the biochemical and morphologic disturbances one would expect that these biochemical changes precede the occurrence of the morphologic changes.

It is difficult to evaluate such a supposed relationship quantitatively, and from the experiments presented in this paper it is only possible to conclude that in thymocytes, myeloid leukemia cells, and C57BL-lymphoma cells the extent of the radiation-induced biochemical disturbances, namely the inhibition of nuclear and mitochondrial oxidative phosphorylation, precedes the extent of recognizable damage (as observed after irradiation in thymus and lymphosarcomatous spleens).

This seems to us a suggestive reason to consider the inhibition of nuclear oxidative phosphorylation in combination with inhibition of mitochondrial oxidative phosphorylation as the principal biochemical lesions known so far associated with interphase death.
CBA-lymphoma cells many abnormal mitoses are seen after irradiation (de Vries, unpublished results).

The results obtained with the F1-lymphosarcoma demonstrate that after irradiation nuclear ATP synthesis is only slightly inhibited in a tissue during a period of complete absence of mitosis. It has been reported previously that no correlation exists between the capacity of cells to perform nuclear oxidative phosphorylation and their mitotic indices (14). However, whether inhibition of nuclear oxidative phosphorylation causes inhibition of mitosis remains an open question.

The differences observed in biochemical radiosensitivity remain unexplained. It seems unlikely that variations in external factors, e.g., oxygen supply, are responsible for the differences observed.

Internal factors like the base composition of DNA have been considered as factors determining radiosensitivity in micro-organisms (13), and radiosensitization in vitro of mouse ascites lymphoma cells has been achieved by a partial replacement of thymine by bromouracil (18). Whether or not the base composition of DNA is the only factor determining radiosensitivity of mammalian cells which die in delayed mitoses is irrelevant to an explanation of radiosensitivity of cells dying shortly after irradiation in interphase. Interphase death is preceded by pycnosis and by serious inhibition of nuclear oxidative phosphorylation followed by inhibition of mitochondrial oxidative phosphorylation. So far, these biochemical lesions have been considered as the major causes of interphase death (14). There is no evidence that DNA is on the direct pathway from the biochemical lesions in interphase. Interphase death is preceded by pycnosis and by serious inhibition of nuclear oxidative phosphorylation followed by inhibition of mitochondrial oxidative phosphorylation. It has been reported previously that no correlation exists between the capacity of cells to perform nuclear oxidative phosphorylation and their mitotic indices (14). However, whether inhibition of nuclear oxidative phosphorylation causes inhibition of mitosis remains an open question.

The differences observed in biochemical radiosensitivity remain unexplained. It seems unlikely that variations in external factors, e.g., oxygen supply, are responsible for the differences observed.

Internal factors like the base composition of DNA have been considered as factors determining radiosensitivity in micro-organisms (13), and radiosensitization in vitro of mouse ascites lymphoma cells has been achieved by a partial replacement of thymine by bromouracil (18). Whether or not the base composition of DNA is the only factor determining radiosensitivity of mammalian cells which die in delayed mitoses is irrelevant to an explanation of radiosensitivity of cells dying shortly after irradiation in interphase. Interphase death is preceded by pycnosis and by serious inhibition of nuclear oxidative phosphorylation followed by inhibition of mitochondrial oxidative phosphorylation. So far, these biochemical lesions have been considered as the major causes of interphase death (14). There is no evidence that DNA is on the direct pathway from the biochemical lesions induced by X-irradiation to interphase death. The fact that nuclear oxidative phosphorylation can only proceed in the presence of DXA does not imply a specific function of DXA in this process, because it can proceed in the presence of other polyanions as well (2). The recent discoveries concerning repair of radiation lesions in micro-organisms by enzymes already present or induced by radiation force us to consider the possibility that analogous mechanisms are operative in mammalian cells. Variations in the activities of such enzymatic repair mechanisms could at least partly explain the variations in radiosensitivity of mammalian cells as they are observed after irradiation in vivo.

Acknowledgments

The histologie preparations were performed by Dr. M. J. de Vries; a full account of his work will be published elsewhere. The experiments with mitochondria were performed by Dr. D. W. van Bekkum and Miss H. T. M. Nieuwerkerk. The authors are indebted to them for permitting the publication of their results in this paper. The valuable discussions with Dr. D. W. van Bekkum are gratefully acknowledged.

References

Fig. 1. Leukemic mouse spleens 4 hr after 900 rads whole-body irradiation. A, Myeloid leukemia; B, C57BL-lymphosarcoma; C, CBA-lymphosarcoma; d, F1-lymphosarcoma. Note absence of mitotic figures. Extensive pycnosis and karyorrhexis in A and B, while the majority of cells is still intact in C and D. H & E, X 300.
Biochemical Radiosensitivity of Lymphoid Tumors

H. M. Klouwen, W. M. Appelman and C. M. J. Arts


Updated version Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/27/2_Part_1/255

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.