Activation of the carcinogen N-hydroxy-2-acetylaminofluorene by rat mammary peroxidase

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Summary. A peroxidase preparation from rat mammary gland parenchymal cells, a target tissue of arylamine carcinogens, was shown to activate the carcinogen N-hydroxy-2-acetylaminofluorene via a nitroxyl free radical intermediate to the more active carcinogens nitrosofluorene and N-acetoxy-2-acetylaminofluorene. Hydrogen peroxide, cumene hydroperoxide, and linoleic acid hydroperoxide were effective as substrates. The antioxidants ascorbate, propyl gallate and reduced glutathione prevented the free radical activation route.

The first step in the activation of the arylamine carcinogen 2-acetylaminofluorene (AAF) is N-hydroxylation to form N-hydroxy-2-acetylaminofluorene (N-OH-AAF). Subsequent proposed activation steps include the free radical mechanism of formation of the more active carcinogens nitrosofluorene (NOF) and N-acetoxy-2-acetylaminofluorene (N-OAc-AAF), a sequence demonstrated by Bartsch et al.[1,2] and Floyd et al.[3–5] for model system peroxidases including horseradish peroxidase, lactoperoxidase, hematin and methemoglobin. This study reports the activation of N-OH-AAF via the free radical mechanism by enzymes of rat mammary gland parenchymal cells, a target cell for arylamine carcinogens[6].

Materials and methods. N-hydroxy-2-acetylaminofluorene (N-OH-AAF) was synthesized from 2-nitrofluorene by the method of Poirier et al.[7]. Linoleic acid hydroperoxide was synthesized according to Hamberg and Gotthammar[8]. Rat mammary parenchymal cells were prepared by the method of Topper et al.[9] and then sonicated at a medium setting for 10 sec. Electron spin resonance spectra were obtained with a Varian E-9 X-band spectrometer in an assay system containing 133 μg/ml N-OH-AAF, 5 mM

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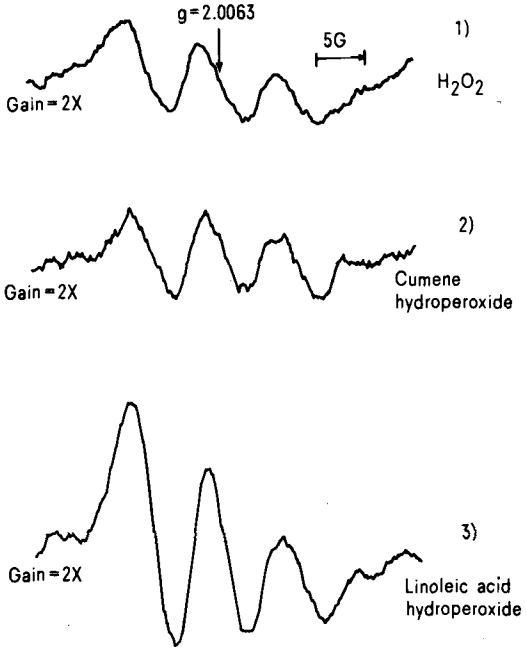

Fig. 1. ESR-spectra of reaction of mammary parenchymal cells, 33 μg/ml N-OH-AAF and 5 mM H_2O_2, 5 mM cumene hydroperoxide, or 0.4 mM linoleic acid hydroperoxide in 50 mM potassium phosphate pH 7.4. All spectra were obtained with 25 mW incident power at a frequency of 9.536 GHz with a modulation amplitude of 5 G, a filter constant of 3 sec and a scan speed of 25 G/min. Temperature was ambient ca. 23 °C.

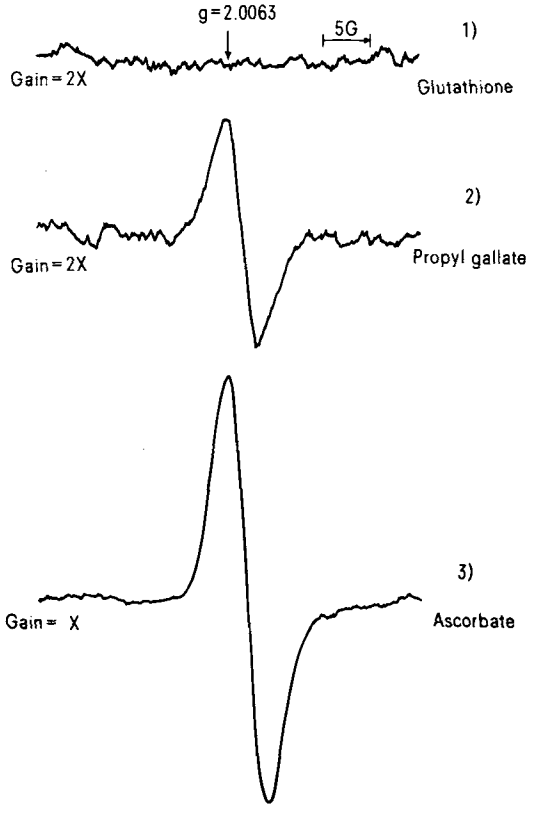

Fig. 2. ESR-spectra of reaction of mammary parenchymal cells, 133 μg/ml N-OH-AAF, 5 mH H_2O_2 and 5 mM reduced glutathione, propyl gallate or ascorbate in potassium phosphate pH 7.4. Same ESR settings as in figure 1.

H_2O_2 or cumene hydroperoxide, or 0.4 mM linoleic acid hydroperoxide and rat mammary parenchymal cell preparations in 50 mM potassium phosphate pH 7.4.

Results and discussion. Figure 1 shows the electron spin resonance (ESR) trace for the reaction of N-OH-AAF with rat mammary peroxidase in the presence of the peroxides H_2O_2, cumene hydroperoxide, and linoleic acid hydroperoxide. A g-value of 2.0063 and a splitting of 7.7–7.9 G characteristic of the nitroxyl free radical of N-OH-AAF were observed. The nitroxyl free radical is the predicted intermediate for the free radical oxidation of N-OH-AAF into NOF and N-OAc-AAF[10]. Formation of NOF and N-OAc-AAF and disappearance of N-OH-AAF were confirmed by thin-layer chromatography on Silica Gel F plates with a dichloromethane-acetone (85:5, v/v) solvent.

Figure 2 shows the effects of the antioxidants ascorbate, reduced glutathione and propyl gallate on the formation of nitroxyl radical. Antioxidant concentrations equal to H_2O_2 concentrations were used. Ascorbate, propyl gallate and reduced glutathione completely eliminated the nitroxyl free radical formed from N-OH-AAF. In the case of ascorbate and propyl gallate an ESR signal was observed as a result of the preferential oxidation of these compounds into their free radical forms. For ascorbate the observed signal is characteristic of the ascorbyl radical (A_H = 1.7 gauss), although for the particular spectrum presented modulation amplitude was large and the characteristic hyperfine structure of this radical was not resolved.

This study suggests that enzymes from a target cell are capable of activating the carcinogen N-OH-AAF into the more active N-OAc-AAF and NOF via a nitroxyl free radical intermediate in a mechanism analagous to that proposed for model system peroxidases. This reaction is inhibited by antioxidants including the physiological compounds, ascorbate and glutathione. Further purification and characterization of the rat mammary peroxidase and studies of the effects of antioxidant levels on the carcinogen activation are currently underway.

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DNA-dependent-DNA-polymerase: Possible limiting influence on cell reproduction during viral leukemogenesis*

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Summary. Evidence is presented that during viral leukemogenesis spleen cell nuclei show an increase in labelling index and mean grain count, that is not accompanied by any changes in the nuclear level of DNA-polymerase-α. It is suggested that polymerase production remains under the control of the normal cell mechanisms and the virus may affect cell proliferation by altering the primer-template levels.

Cancer development in both humans and animals is often characterized in the initial growth phase by a population of rapidly proliferating cells and in the later growth phase by a large compartment of slow growing or resting, G_0, cells[1]. Previous studies in our laboratories have shown this also to be the case in mice given Rauscher leukemia virus, RLV[2–4], for which the hematopoietic stem cell has been identified as a leukemia virus target cell[5–7]. In these mice, even though the first response to the virus is to increase the number of DNA synthesizing (S-phase) cells[3,4], measurement of the splenic stem cell G_0 compartment at the 14th day after virus administration has shown it to be 20 times the normal size for a non-leukemic control[2]. Since at the same time, and at still later disease stages, Rauscher leukemic mice are also known to have massive serum virus titers[8], it can be inferred that although RLV does initially increase the number of proliferating S-phase stem cells it cannot of itself maintain an increased rate of proliferation in the target stem cells, even at these high virus titers. Hence, the possibility is suggested that there may be a limiting necessary factor for DNA synthesis, even in the leukemic cell, which cannot be influenced by the virus.

To further investigate this possibility in the Rauscher leukemia model we have utilized the primer-dependent-polymerase (PDP) assay technique recently developed by Schiffer et al.[9]. This technique allows one to study not only the changes in the fraction of proliferating cells during leukemogenesis through DNA labelling, but further to discriminate within the labelled populations as to whether the changes in labelling are a consequence of changes within the cell nucleus in DNA-dependent-DNA-polymerase concentration, or in primer-template, or both[10].

Materials and methods. The animals used were female SJL/J mice injected with 50 $SED_{50/14}$ units of RLV[2–4] at 12 weeks of age. As controls normal, non-injected, mice selected from the same lots were used. To allow for correlation of the present studies with our previous G_0 studies[2] the mice were sacrificed 14 days after RLV injection. The spleens were removed, single cell supensions prepared in Hank's solution supplemented with fetal calf serum, and dry film smears prepared on acid cleaned

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