DNA Repair Synthesis following Exposure to Chemical Mutagens in Primary Liver, Stomach, and Intestinal Cells Isolated from Rainbow Trout

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ABSTRACT

DNA repair synthesis was autoradiographically measured in liver, stomach, and intestinal cells isolated from rainbow trout which were exposed in vitro to the chemical mutagens, N-methyl-N' nitro-N-nitrosoguanidine, 4-nitroquinoline 1-oxide, and aflatoxin B1. The level of repair was greatest in primary hepatocytes which responded to all three mutagens. Only nominal amounts of repair were detected in stomach cells following N-methyl-N' nitro-N-nitrosoguanidine and 4-nitroquinoline 1-oxide exposures and in intestinal cells following 4-nitroquinoline 1-oxide exposure. In comparison with cultured rainbow trout cells, the quantity of DNA repair found in primary cells is significantly less.

INTRODUCTION

In chemical carcinogenesis testing with aquatic animals, considerable use has been made of the rainbow trout, due to its economic importance, large resource literature, and the ease of acquiring species strains found to be extremely sensitive to some carcinogens, notably AFB1. Payne et al. (6) and Stott and Sinnhuber (9) have shown that rainbow trout S9 can activate aflatoxin metabolites to DNA in isolated hepatocytes from rainbow trout. In humans, the condition xeroderma pigmentosum is a failure to repair DNA lesions, which predisposes the individual to cancer (3). Xeroderma pigmentosum cell cultures exhibit very little DNA repair following exposure to chemical carcinogens (8). Phylectic comparisons show that cultured fish cells from rainbow trout have very little unscheduled DNA repair activity (10). This low activity may result from a reduction or loss of excision repair during prolonged culture of cell lines, as with the mouse fibroblast 3T3 line (2).

In order to examine the possible loss of DNA repair synthesis in cultured rainbow trout cells, we have measured repair synthesis in freshly isolated rainbow trout liver, stomach, and intestinal cells following exposure to MNNG, 4NQO, and AFB1.

MATERIALS AND METHODS

Isolation of Primary Cells. Liver, stomach, and intestine were removed from 150- to 200-g rainbow trout. Using a syringe, the stomach and intestine were flushed with cool, sterile BSS supplemented with antibiotics (polymyxin B, 83.3 mg/liter; bacitracin, 800 mg/liter; neomycin, 5 mg/liter). The liver was rinsed with BSS and then minced with 5 ml BSS in a sterile plastic Petri dish (Falcon Plastics). The stomach and intestine were cut longitudinally to expose the mucosa. Each tissue was placed into a sealed sterile polycarbonate Erlenmeyer flask containing 10 ml BSS with Pronase (0.5%), and incubated at 20° for 45 to 60 min with occasional agitation to loosen the cells. Following the incubation period, the solution was pipetted off, and the remaining tissue was washed with BSS. The enzyme solution and washings were pooled and passed through a piece of sterile gauze to remove large tissue pieces. The cells were centrifuged at 600 rpm for 5 min, resuspended in 6 ml 2.5% ADM, and then centrifuged and resuspended in fresh 2.5% ADM.

Chemicals. Chemicals were obtained from the following sources: MNNG and 4NQO from Aldrich Chemical Co., Milwaukee, WI; AFB1 and Pronase from Sigma Chemical Co., St. Louis, MO; [3H]dThd (specific activity, 25 Ci/mmol) from Amersham Corp., Amersham, England.

DNA Repair Synthesis. [3H]dThd was diluted with 2.5% ADM (final working concentration, 10 μCi/ml), and this medium was used for the mutagen dilutions. 4NQO and AFB1 required initial dissolving in dimethyl sulfoxide (final concentration, <1%) prior to dilution with medium, while MNNG dissolved directly into the 2.5% ADM. Aliquots (1 ml) of cell suspension and mutagen:[3H]dThd were added to 3.5-cm plastic Petri dishes, which were placed in a sealed CO2-flushed container at 18° for 6 hr.

At the end of the exposure period, the cells were pipetted into test tubes, centrifuged, and resuspended in ethanolic acetic acid (3:1). After 10 min, the cells were again centrifuged; then all but approximately 0.2 ml of the fixative was removed. This remaining fixative was used to resuspend the cells, which were dropped onto microscope slides. Air-dried slides were coated with nuclear track emulsion (NTB-3, Eastman Kodak, Rochester, NY) and kept in light-tight boxes at 4° for 30 days. DNA repair synthesis was measured as the mean number of silver grains over at least 30 nuclei for each data point.

RESULTS AND DISCUSSION

The viability of all cells was tested at 2 and 6 hr, using the trypan blue exclusion technique. Observed mortality was generally less than 5 to 10% after 6 hr.

The level of DNA repair (Chart 1) is greatest in primary liver cells for the 3 chemical mutagens tested. The magnitude of response is greatest with 4NQO and least with AFB1. A longer period for AFB1 activation may have produced a greater repair response, but Bailey et al. (1) have demonstrated a decrease in the rate of AFB1-DNA formation beyond 3 hr in rainbow trout hepatocytes; if the rate of DNA repair is similar to that of cultured rainbow trout cells (10), then a significant amount of repair should have been detected within the 6-hr exposure period used here.

Stomach cells show small amounts of repair in response to MNNG and 4NQO but fail to respond to AFB1. Intestinal cells exhibit a small amount of repair following exposure to 4NQO only.

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2 To whom requests for reprints should be addressed.
3 The abbreviations used are: AFB1, aflatoxin B1; [3H]dThd, [3H]thymidine; MNNG, N-methyl-N' nitro-N-nitrosoguanidine; 4NQO, 4-nitroquinoline 1-oxide; BSS, Hank's balanced salt solution, pH 7.4; 2.5% ADM, arginine-deficient medium supplemented with 2.5% fetal calf serum.
These generally low amounts of DNA repair in primary fish cells, following exposure to chemical mutagens, are paralleled by similar findings for fish cells by Woodhead et al. (11) and Walton et al. (10) in vitro and by Ishikawa et al. (5) in vivo. Despite using assay conditions found to enhance grain production in cultured rainbow trout cells, the DNA repair synthesis in the primary cells examined here is less than expected from comparable exposures for cultured rainbow trout cells (10). Unlike mouse cells (2), where a loss of excision repair has been noted through time in culture, it appears that prolonged culture of cells from rainbow trout tissues does not result in a loss of excision repair but rather in a maintenance and possible enhancement of such repair.

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