THE EFFECT OF TRIETHYLENE MELAMINE ON DNA SYNTHESIS AND MITOSIS IN THE LENS EPITHELIUM

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INTRODUCTION

The alkylating antitumor agent, triethylene melamine (TEM), is well known to ophthalmologists because of its successful combination with X-irradiation in therapy of retinoblastoma. REESE and coworkers (1955) selected this compound of the ethylene imine group because it can be administered orally and may attack embryonic retinal tissue in a manner similar to that demonstrated with nitrogen mustard in the developing amphibian eye (GILETTE & BODENSTEIN, 1946). Inhibition of mitosis and cell death are the obvious morphological signs of the effects of ethylene imines. It is not clear whether this cytotoxicity is brought about by alteration of deoxyribo-nucleic acid (DNA), although the reaction of these compounds with DNA and pyrimidine precursors has been demonstrated in vitro and in vivo (ALEXANDER et al., 1959; TRAMS et al., 1961; LORKIÊQICZ & SZYBALSKI, 1961). Moreover, there is little information on the action of TEM and related compounds on the life cycle of cells.

Flat mounts of the lens epithelium provide favorable conditions for such investigations as shown in previous studies from this laboratory. The duration of the various phases of the normal cell cycle has been established* and recently, differences in the mode of action of ionizing radiation, myleran, and TEM in

* Cells of the rat lens epithelium normally require 16 hours to pass from the beginning of DNA synthesis to the completion of mitosis. This interval is partitioned as follows: DNA synthesizing (S) period, approximately 10 hours; post-synthetic (G2) period, close to 5 hours; and mitosis, 72 minutes. The cells are segregated into regions characterized by different durations of the pre-DNA synthesizing (G1) period and, consequently, of the intermitotic time. In young rats cells of the central area have an approximate intermitotic time of over 250 days; those of the pre-equatorial zone, 31 days; and those of the equatorial zone, 19 days. The differentiating cells in the extreme periphery, in the meridional rows, do not undergo division.
this experimental system have been briefly described (v. SALLMANN, 1965).

The present communication reports in detail the effect of a single injection of TEM on DNA synthesis, mitosis, and the incidence of cell death in the lens epithelium of young rats.

MATERIALS AND METHODS

Young male rats (160-180 g) of the Osborne-Mendel strain were used. A total of 107 animals were injected with TEM intravenously in a dose of either 1 or 2 mg/kg, and 30 untreated rats served as controls. The drug solution was always freshly prepared in a concentration of 1 mg/cc. At selected time intervals (Table I) groups of TEM-treated rats and control animals received an injection of H\textsuperscript{3}-thymidine (1 \mu c per gram of body weight i.p.) and were killed one hour after administration of the isotope. The specific activity of the tracer compound was 3.0 curies per millimole (Schwartz Bioresearch Inc.).

The eyes were removed, incised near the posterior pole, and fixed for 24 hours in a solution of three parts 95% ethanol and one part glacial acetic acid. Feulgen-stained flat mounts of the lens epithelium prepared as described previously (SCULLICA et al., 1962) were covered with Kodak AR-10 stripping film and exposed for 40 days.

The entire cell population of each lens epithelium preparation was examined at a magnification of 500 times and the total number of mitoses, H\textsuperscript{3}-labeled nuclei, and degenerate nuclei was determined. The counts of all control animals killed during the first week were grouped together to give a single average value, as no significant differences were detected between specific intervals within this period. At two weeks and later, average control values were calculated for each interval.

The mean grain count was established by recording the number of exposed silver grains over 100 labeled nuclei in each preparation at a magnification of 1250 times. The DNA content of individual nuclei was determined in some preparations by measuring the light absorption of the Feulgen-DNA complex with an integrating microdensitometer (DEELEY et al., 1954).

RESULTS

Rats injected with TEM in a dose of 1 mg/kg showed mild signs of gastrointestinal toxicity and failed to gain weight during the week following treatment, but almost all animals survived this period. Thereafter, they grew normally and remained in good condition up to the end of the observation period at 8 weeks.