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COMPARATIVE STUDY OF THE EFFECTS OF ETHIDIUM BROMIDE AND DNA-ETHIDIUM BROMIDE COMPLEX ON NORMAL AND CANCER CELLS

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We have recently studied the antimitotic effect of ethidium bromide (EB) on normal and tumour cells cultivated in vitro (1, 2). As this drug is strongly bound by DNA (3), it was of interest to compare the effect of free and of a DNA-EB complex. Indeed, Trouet et al., (4) have shown that drug uptake by tumour cells can be enhanced when the drug is associated with a " non-permeant, pinocytosable and digestible carrier (lysosomotropic drug) " as many tumour cells are characterised by a high endocytic activity. These authors have successfully used a DNA-daunorubicin complex against mouse and human leukemia.

We analyse here mainly by cytological and cytochemical methods (as EB can be detected by fluorescence microscopy and differenciation can be obtained between free and DNA bound EB), the toxicity of EB and DNA-EB in chick embryo fibroblasts cultivated in vitro and in Ehrlich tumour cells either cultivated in vitro or inoculated into mouse peritoneum. Calf thymus DNA (type V, Sigma) was dissolved in sterile 10^{-3}M NaCl. Mixtures with ethidium bromide (Calbiochem) were made in sterile 0.1 M NaCl; the relative final concentrations assayed were : 0.15 mgr EB with 0.5 mgr/ml DNA and 1 mgr/ml EB with 1.8 mgr/-

ml DNA (determined by P content).

Chick embryo fibroblasts were cultivated in vitro and EB or DNA-EB was added to the culture medium. EB (5 µg/ml) provokes mitochondrial swelling and nucleolar segregation (phase contrast observations of living cells), completely inhibits cell multiplication after 48 hours and blocks many cells in the S phase of the cycle (absorption cytophotometry after Feulgen reaction). After treatment by DNA-EB (5 µg/ml EB), the cytotoxicity is much lower and the alterations are reversible. EB rapidly diffuses into the cytoplasm and nucleus (fluorescence microscopy) but DNA-EB is very slowly absorbed by the cells and first appears in vacuoles. Macrophages very actively absorb the complex.

Hypertetraploid Ehrlich tumour cells (ELT) growing in vitro were treated by EB (5 µg/ml) or DNA-EB (5 µg/ml EB). Under these two experimental conditions, cell multiplication is inhibited; mitochondrial swelling and nucleolar segregation have been observed. The latter cellular alteration appears earlier (6 hours) after DNA-EB treatment than after EB treatment (24 hours). Chromatin, nucleoli and cytoplasm are fluorescent 15 min. after EB treatment; DNA-EB first appears in the cytoplasm after 15 min. and afterwards in the nucleus. After 1 hour, fluorescence is identical in EB or DNA-EB treated cells.

EB concentration can be measured in the blood by spectrophotometry, at least for concentrations higher than 10 µg/ml. After intraperitoneal injection into adult rats, DNA-EB can be detected in the blood. However, the passage of EB from the peritoneal cavity into the blood

stream is more rapid. After intraperitoneal injection of EB or DNA-EB, fluorescence can be detected in endothelial and muscle cells of the peritoneal vessels.

EB or DNA-EB was injected into the peritoneal cavity of C 57 Bl mice bearing an hypertetraploid Ehrlich ascites tumour (1 injection of 1 mg EB). In tumour cells, the toxicity of DNA-EB is higher than that of EB (inhibition of cell multiplication, nucleolar segregation, mitochondrial swelling and intramitochondrial inclusions). EB and DNA-EB are detectable inside the tumour cells one hour after the injection. Control experiments have shown that DNA alone has no or only a slight toxic effect in our cellular material.

In conclusion of this preliminary work, it appears that the cytotoxic effect of DNA bound EB is qualitatively the same as that of free EB. However : 1° the localization of the bound drug early after treatment is different : it is detectable in cytoplasmic vacuoles; also, it penetrates into normal cells and through blood vessels more slowly than free EB; 2° in Ehrlich tumour cells, the cytotoxic effect of DNA bound EB is more pronounced and appears earlier after treatment. Thus, the level of cellular toxicity we have observed clearly depends upon the mode of penetration of the drug; this confirms, in the case of ethidium bromide, the idea of Trouet et al., (4).

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