DRUG-INDUCED MITOCHONDRIAL PROLIFERATION

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Although proliferation of mitochondria has been noted in the liver of patients suffering from a number of illnesses, the relation of this phenomenon to the disease is not known. Several liver carcinogens are known to induce mitochondrial proliferation shortly after administration; but again, the relationship to hepatocarcinogenesis is not known. Several carcinogens having this effect are not detectably mutagenic, suggesting that their effect on mitochondria might be related to carcinogenesis. The most notable mitochondrial-proliferation carcinogens are methapyrilene (formerly a commonly used antihistaminic), diethylhexylphthalate (DEHP; a widely used plasticizer), nitrosodiethanolamine and nitrosomethylethanolamine (both contaminants of cutting oils and cosmetics). Methapyrilene induces liver tumours in rats, but not in other species, and several structurally close analogues are not carcinogenic. These analogues do not induce mitochondrial proliferation, nor does methapyrilene in species other than the rat.

Proliferation of mitochondria is not a very common situation in cells, but has been noted in the liver of various hospitalized patients: in about one-third there was an association with chronic hepatitis and cirrhosis [1], but in the others there was no association with a particular disease. The analysis, incompletely described, was based on assessment of the numbers of mitochondria in serial sections examined by electron microscopy (e.m.). The mitochondria were normal in size and in cristal configuration, whilst greatly increased in number. The authors discussed the association between hypoxia and mitochondrial proliferation, as also manifest in nutritional deficiencies of vitamin E and copper. The mechanism of this phenomenon is not known, nor its significance in disease.
Scrutiny of the pathological effects of carcinogens, e.g. azo dyes, has often revealed ultrastructural changes. The non-carcinogenic azo dye, 2-methyl-4-dimethylaminoazobenzene, a close analogue of 3'-methyl-4-dimethylaminoazobenzene which induces liver tumours in rats, caused an increase in the mitochondrial content of rat liver cells, besides other changes [2]. More recently, an ultrastructural study of the action of methapyrilene hydrochloride showed that it produced extensive proliferation of mitochondria in the liver cells of rats [3], the target cells of this carcinogen.

Methapyrilene was one of several antihistaminic drugs that were tested for carcinogenic activity because they were capable of reacting with nitrosating agents to form carcinogenic nitrosamines [4]. It induced a very high incidence of hepatocellular and cholangiocellular neoplasms in rats, when fed for a year or more at 1,000 ppm; the concurrent feeding of sodium nitrite did not change the results, indicating that the formation of liver tumours was not related to nitrosation of methapyrilene [5]. This compound appears to act as a carcinogen through an unconventional mechanism, since it is not a mutagen in bacteria [6] nor in other mutagenic systems, even when activated by a rat liver microsomal preparation. Nor is it active in the Syrian hamster embryo transformation assay, even when activated [7]. The compound does not appear to be 'genotoxic', i.e. it does not cause a structural change in cellular DNA. There is no suggestion of chromosome damage induced by methapyrilene: searches for sister-chromatid exchanges in rat liver, *in vitro* or *in vivo*, have failed [8].

**POSSIBLE BINDING OF METHAPYRILENE TO CELLULAR MACROMOLECULES**

In accord with the lack of chromosome damage, a study of the interaction of radiolabelled methapyrilene with cellular macromolecules showed negligible binding to DNA and RNA in the liver of rats given a single dose of 20 mg (³³H ~ 1 mCi)[9]. There was extensive binding to liver proteins, but the specific activity of the nucleic acids was infinitesimal in liver, as for other rat organs - e.g. lung and kidney - which are not target organs for the carcinogenic action of methapyrilene. In the same experiment, radioautography of the livers at 1, 6, 14, 24 and 44 h after treatment showed that the maximum radioactivity was at 6 h [3], coinciding with the maximum binding observed chemically [9]; binding remained substantial 44 h after treatment. At each time, 50 hepatocytes from the periportal region and 50 from the centrilobular region were examined; most radioactivity was in the former. Other cells in the liver were not labelled.

Radioautography by e.m. gave values (as % of total grains/cell) for radiolabel distribution within periportal hepatocytes at 6 h:
- nucleus (10% of cell area) and nucleolus (0.5%) each nil;
- mitochondria (7.5% of cell area), 67%;
- endoplasmic reticulum (e.r.): rough (22%), 9%; smooth (46%), 23%;
- lipid (2.0%), 0.9%; lysosomes (1.4%), 0.2%.