Effects of Prednisolone on the Differentiation of Mouse Lung Adenomas in Culture

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INTRODUCTION

The urethan-induced pulmonary adenoma is a standard model of tumorigenesis (10). Although the tumor cells bear a morphological resemblance to type II alveolar epithelial cells of normal lung (2), little attention has been paid to the consequences of this finding, and in fact we are aware of only 2 studies that have examined directly the function of these tumors (7, 12). In each, it was apparent that, despite their neoplastic nature, the lung adenomas were capable of synthesizing the phospholipids of pulmonary surface active material, a property of type II cells in the normal lung.

Glucocorticoid hormones are believed to play a role in the initiation of surfactant synthesis in the fetal lung. These steroids affect cellular differentiation in the developing lung by slowing cell division and, conversely, accelerating cell maturation (5). Glucocorticoids probably act on type II cells since steroid treatment alters phospholipid metabolism and epithelial cell morphology in fetal and adult lung in vitro (1) and in vivo (8, 16). In an effort to extend the analogy between type II cells and the cells comprising this tumor, we have tested the effect of prednisolone on urethan-induced adenomas in an organ culture system.

MATERIALS AND METHODS

Tumor Induction. Female Swiss-Webster mice, between 4 and 6 weeks of age, were given i.p. injections of 1 mg of urethan per kg (ethyl carbamate; British Drug Houses, Poole, England). Large tumors were dissected from the pleural surface of the lungs between 12 and 16 months after the single injection. The following procedures of organ culture and of morphological and biochemical examination were carried out on a series of tumor samples from 5 different mice, whose lungs bore multiple tumors.

Culture Methods. With the use of aseptic techniques, 1-cm-mm pieces of tumor were cut and placed in the wells of Teflon culture chambers (1). From each mouse sacrificed, 2 chambers were prepared with standard T8 medium (15) and 2 chambers with prednisolone (1 μg/ml) added to the T8 medium. The tumor explants were then cultured for 72 hr at 37 ± 1° with a constant gas flow of 95% O2-5% CO2. Some of the remaining tumor pieces were prepared for light and electron microscopy. After culture, several pieces of the tissue were fixed in glutaraldehyde, postfixed in osmic acid, dehydrated, and embedded in Spurr. Thick sections (0.5 μm) were examined by light microscopy, and ultrathin sections were cut and stained with lead citrate for electron microscopy. The remaining pieces of the cultured tumors were removed and rinsed with fresh culture medium prior to the isotopic uptake studies.

Biochemical Methods. Three-tenths ml of a solution containing 5 μCi/ml [14C]palmitic acid complexed with albumin was added to a fresh 10-ml aliquot of medium containing the cultured samples. This mixture was incubated for 1 hr at 37° in a shaking incubator, after which the tissue was removed, rinsed, and extracted in chloroform-methanol to obtain the lipid fraction. The lipid separations were performed as previously described (7). The phospholipid fraction was obtained using Florisil chromatography (4) with 3 elution steps: (a) chloroform to remove neutral lipids, (b) 15% methanol in chloroform to remove most phospholipids other than phosphatidylcholine and sphingomyelin, and (c) pure methanol to remove the latter phospholipids. In these tumors, the amount of sphingomyelin in the methanol fraction is small compared to the amount of phosphatidylcholine. The final fraction was then adducted with mercuric acetate according to Mangold’s (9) technique, and the SPC fraction was isolated by thin-layer chromatography. With this method, the recovery of a purified sample of [14C]-labeled dipalmitoyl lecithin (Applied Science Labs., State College, Pa.) is greater than 75%. Gas-liquid chromatography confirmed that the saturated phosphatidylcholine fraction does in fact contain >95% saturated fatty acids, most of which are palmitate. Standard methods were used for the DNA and phosphorus determinations and for scintillation counting.
RESULTS

Morphology. The pleural surfaces of the urethan-injected mice were covered by readily recognizable tumors after 6 months, and the size of these tumors increased with time thereafter to become quite large at 1 year (Fig. 1). Microscopically, the tumors were poorly vascularized and showed tubular structures lined by cuboidal cells. All tumors were noninvasive and histologically were classed as adenomas. The observed slow growth rate of this tumor was substantiated by the failure to observe mitotic figures in any of the microscopic sections.

After 3 days of culture without steroid, the tumor morphology did not change and there was no difference between the cultured and noncultured tumor samples (Fig. 2a). By electron microscopy, the cuboidal epithelial cells contained a few lamellar bodies in the cytoplasm (Fig. 3) and generally resembled adenoma cells described previously (2, 7).

There was a significant morphological change in the tumor cells after 3 days of culture in the presence of prednisolone. By light microscopy, the structure of the tumor was unaltered but the cells contained many vacuoles (Fig. 2b). By electron microscopy, it was shown that these structures correspond to lamellar bodies. The tumor cells after steroid treatment appeared to contain many more lamellar bodies than control tissue and bore an even more striking morphological similarity to the type II cell of normal lung (Fig. 4).

The number of lamellar bodies per cell was counted on a random sample of 100 tumor cells incubated with or without steroid. Control cultures had a mean of 2.5 lamellar bodies per cell, whereas prednisolone-treated tumor cells contained a mean of 11.8 lamellar bodies per cell.

Biochemistry. Five separate pairs of incubated tumor samples were studied, each specimen being analyzed in duplicate. Chart 1 illustrates the percentage change in several biochemical measurements following steroid treatment. Although there is an apparent difference in uptake of phospholipid precursors, the differences are not statistically significant. Overall, the mean incorporation of [14C]palmitate in SPC3 was unchanged (steroid, 13.3 x 103 ± 2.9 SE; control, 11.3 x 103 ± 2.6 in cpm/g fresh weight). In addition, no significant change was seen in the specific activity of [14C]palmitate in the SPC fraction (steroid, 1.6 x 105 ± 0.1; control, 1.3 x 105 ± 0.2 in cpm/mg SPC). Between steroid-treated and control samples there was no detectable difference in DNA content, (steroid, 8.9 ± 1.1; control, 9.0 ± 1.4 mg/g) or in SPC content (steroid, 8.8 ± 2.3; control, 8.5 ± 1.8 mg/g).

DISCUSSION

The observation that tissue incubated for 3 days without steroid had the same appearance as fresh tumor led us to conclude that the explant system was successful in maintaining a healthy tissue status, and that the changes found in the steroid experiments were attributable to the presence of prednisolone in the culture medium.

Lamellar bodies in type II alveolar cells have traditionally been considered as analogous to "secretory granules" containing pulmonary surfactant (3). Their increased number in a variety of physiological and pathological conditions has been interpreted as evidence of enhanced surfactant secretion (6), and the initial appearance of lamellar bodies in the type II alveolar cell of the developing fetus is regarded as an index of epithelial maturity.

The finding of some lamellar bodies in urethan-induced lung adenomas suggested that these tumors were derived from type II alveolar cells and that their function might in part be preserved. There is biochemical evidence to substantiate this impression (7, 12). Glucocorticoids have been shown to accelerate the appearance and increase the number of lamellar bodies in studies of fetal lung performed in vivo and in vitro (1, 8, 16). In the present experiments, prednisolone treatment of adenomas resulted in an increase in the lamellar body content of the tumor cells to the extent that these cells became indistinguishable from normal, active type II cells of mammalian lung (2, 6, 13, 16).

The differentiation of the tumor cells into morphologically mature type II cells was not accompanied by a statistically significant change in [14C]palmitate uptake. In many instances, increases in phospholipid metabolism have been demonstrated in association with increased numbers of lamellar bodies (6). The failure to demonstrate increased palmitate uptake may be explained by the short time period over which the uptake was measured or by the insensitivity of this particular assay. For example, if the hormone stimulated the synthesis of a surfactant component other than disaturated lecithins, the palmitate uptake might not be altered. It is also possible that synthesis of saturated phospholipid by these cells is little changed, whereas the secretory process is altered, resulting in an accumulation of the cytoplasmic lamellar inclusions.

Although the effects of prednisolone on pulmonary cell maturity are now under study, the mode of action at the molecular level is, as yet, unknown. Both in vivo and in vitro studies of glucocorticoid have shown that these drugs inhibit DNA synthesis and accelerate maturation processes. It is not known however, whether they exert this influence by a direct stimulus to cellular differentiation, or by first in-

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3 The abbreviation used is: SPC, saturated phosphatidylcholine.
Steroid-treated Lung Adenomas

hibiting cellular division, thereby allowing a consequential acceleration in the maturation process. In the present system, the slow mitotic rate of the adenoma cells suggests that the drug may be acting directly to stimulate cellular maturation of the relatively undifferentiated cuboidal cells of the tumor.

A recent study by Stoner et al. (14) examined the effects of hydrocortisone on clones of epithelial cells derived from lung adenomas. They found that steroid enhanced the growth rate of the tumor cells in an 8- to 10-day culture period; no effect was seen in the 1st 3 days, the time-span of this study. Smith et al. (11) demonstrated a growth-stimulating effect of steroid on cultures of fetal lung prepared when epithelial cells were in a growth phase. However, in cultures prepared from more mature fetal lung, cortisone decreased cellular growth and enhanced maturation, changes similar to those seen in the present study of lung adenomas. It seems apparent, therefore, that not only do steroids affect cellular growth and maturation but that their effect is dependent upon the initial state of differentiation of the tissue. The similarity between the cortisol effect we have observed in the mouse adenoma system and that found in the maturing fetal lung tissue suggests a further parallel between these tumor cells and the developing type II alveolar epithelial cell.

REFERENCES


MAY 1976
Fig. 1. Pulmonary adenomas on mouse lung 1 year after a single injection of urethan.

Fig. 2. Sections (0.5 μm) of tumor cultured for 3 days. a, control: tumor is mainly composed of cuboidal epithelial cells; b, with prednisolone: the epithelial cells now contain many lipid vacuoles. Toluidine blue, × 1000.
Fig. 3. Electron micrograph of control adenoma cultured for 3 days. Cuboidal epithelial cells contain a few lipid inclusions. × 9000.

Fig. 4. Electron micrograph of adenoma cultured for 3 days with prednisolone. Cuboidal cells contain many lamellar bodies. × 9000.
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