Metabolic Activation and Cytotoxicity of 4-Ipomeanol in Human Non-Small Cell Lung Cancer Lines

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ABSTRACT

In the normal lungs of many animal species, 4-ipomeanol is transformed to a highly reactive metabolite preferentially in pulmonary bronchiolar Clara cells and to a lesser extent in alveolar type II cells, potentially leading to damage or destruction of these cell types. Since Clara cells and type II cells are suspected sites of origin of certain “non-small cell” lung cancers, the metabolic activation of 4-ipomeanol (measured by the metabolism-dependent covalent binding of 4-ipomeanol to cellular macromolecules) was compared in two human non-small cell carcinoma derived cell lines (NCI-H322 and NCI-H358) and two human small cell carcinoma derived cell lines (NCI-H128 and NCI-H69). Metabolic activation of 4-ipomeanol was evident in the non-small cell lines; the production of covalently bound metabolite was somewhat greater in NCI-H322 (morphology related to Clara cells) compared to NCI-H358 (morphology related to alveolar type II cells), but was entirely undetectable in the small cell lines. The activation pathway was concentration (4-ipomeanol) and time dependent and followed Michaelis-Menten kinetics. Metabolism to the reactive intermediate required oxygen and was strongly inhibited by carbon monoxide. Covalent binding was enhanced in the non-small cell lines by prior incubation with β-naphthoflavone and by supplementation of the incubate with exogenous reduced nicotinamide adenine dinucleotide phosphate. 4-Ipomeanol was more cytotoxic to the non-small cell lines than to the small cell lines under the in vitro growth conditions used. These studies indicate that certain human non-small cell lung cancers have metabolic characteristics of normal bronchiolar Clara cells and alveolar type II cells; these results would therefore be consistent with an origin of these tumors from Clara cells or type II cells, respectively. The present studies indicate that the further preclinical testing and development of 4-ipomeanol is warranted, with a view toward possible clinical evaluation against human lung cancers.

INTRODUCTION

The natural product, 4-ipomeanol (Fig. 1) was initially isolated and characterized in the early 1970s from sweet potato roots infected with the common fungus, Fusarium solani (1, 2). The discovery of IPO2 provided an explanation for occasionally reported outbreaks of a peculiar and often fatal respiratory disease in cattle and other domestic animals fed mold-damaged sweet potatoes. The subsequent development of a simple chemical synthesis for IPO provided bulk material and radiolabeled derivatives necessary for extensive studies of the chemistry and mechanism of action of this unusual compound (3, 4). IPO has been investigated in a variety of experimental animals and systems in vivo and in vitro, and detailed reviews of many of these studies are available (e.g., see Refs. 5–10). 4-Ipomeanol has served as a key prototype experimental agent in investigations that have elucidated new general mechanisms of drug-induced acute lung injury and have raised important implications for the pathogenesis and therapy of human lung cancer.

IPO is a potent lung toxin in many animal species, the pulmonary Clara cell being a predominant target (11). The compound has been shown to undergo metabolic activation in situ in the lung by a mixed-function oxidase enzyme system (11–13). The high relative overall abundance of this MFO system in Clara cells as well as the involvement of specific cytochrome P-450 isoforms not predominant in other tissues such as the liver may be major factors rendering this cell type more susceptible than other lung cells to IPO (5, 8, 10–12, 14–16). Accordingly, high levels of a covalently bound IPO metabolite are detectable in the rat, mouse, and hamster pulmonary Clara cells by autoradiography after in vivo administration of the radioactive compound (11, 13). Freshly isolated rabbit Clara cells metabolically activate the compound in vitro to a greater extent than do other pulmonary cell populations (17).

Studies with IPO first led to the conclusion that the bronchiolar Clara cells are a major cellular locus of a specialized cytochrome P-450 mixed-function oxidase system in lung (11). The presence of this enzyme system indicated that these cells were also potential targets for other kinds of toxins requiring metabolic activation by the same MFO system. The view was also put forward that the Clara cell could be a potential site of origin of carcinogen-induced bronchogenic lung cancers (11). It is generally accepted that most human lung cancers are carcinoma induced and that many if not most of the major chemical carcinogens require activation by MFO-mediated metabolism. A similar view that Clara cells could serve as progenitors for certain chemically induced lung cancers has evolved from morphological and ultrastructural observations (18). There is also evidence that Clara cells serve as the major stem cell population for the continuing renewal of the normal bronchiolbronchial epithelium (19); therefore, it would not be surprising if indeed these cells would be particularly susceptible to neoplastic transformation. Based on the above observations, the further hypothesis was raised that bronchogenic lung cancers might be uniquely susceptible to bronchiolar epithelial toxins such as IPO, particularly if the tumors retained key susceptibility determinants of their normal bronchiolar or bronchial cell counterparts (11).

IPO provided an ideal prototype agent to further pursue both of the above hypotheses, i.e., as the substrate to probe Clara cell-related metabolic activities of tumors and to examine the in vitro antitumor potential of a known selective lung cell poison against appropriate lung cancer subtypes. The feasibility of such studies and their extension to human tumor materials was greatly facilitated by the availability of well-characterized human lung cancer lines in established cultures. We have therefore investigated the metabolic activation and toxicity of IPO in two selected human non-small cell lung cancer lines, one (NCI-H322) with ultrastructural features of Clara cells (20) and the other (NCI-H358) with ultrastructural features of alveolar type II cells (20). For comparison, IPO was likewise tested in 2 small selected cell lines (NCI-H128 and NCI-H69) which are rich in endocrine-related metabolic activity (20) and which are derived from human small cell lung carcinomas.

MATERIALS AND METHODS

Chemicals. Unlabeled 4-ipomeanol and [3H]ipomeanol (specific activity, 2 Ci/mmol; radiochemical purity, >98%) were prepared as

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2The abbreviations used are: IPO, 4-ipomeanol (1-[3-furyl]-4-hydroxypentanone; NSC 349438); MFO, mixed-function oxidase; FBS, fetal bovine serum; BN, β-naphthoflavone; DMSO, dimethyl sulfoxide; NCI, National Cancer Institute.
previously described (2–4). RPMI 1640 and FBS were obtained from Biofluids (Rockville, MD) and Hy Clone Laboratories (Logan, UT), respectively. NADPH was obtained from Sigma Chemical Company (St. Louis, MO) and 7-ethoxycoumarin and β-naphthoflavone were from Aldrich Chemical Company (Milwaukee, WI).

Preparation of Cells for Incubation. The cell lines used in these studies were developed and kindly provided by the laboratories of Drs. A. Gazdar, J. Minna, and colleagues at the National Cancer Institute. The 2 cell lines derived from bronchioloalveolar carcinomas (NCI-H322 and NCI-H358) which grow as monolayers were maintained in RPMI 1640 supplemented with glutamine (2 mm), FBS (10% v/v), and gentamycin (50 μg/ml) at 37°C in an atmosphere of 95% air/5% CO2. They were subcultured at a density of approximately 1.2 × 106/cm2, and the spent medium was replaced by fresh medium on days 1 and 4. The cells were used after 6–8 days when the monolayer had reached about 75% confluency. The 2 cell lines derived from small cell carcinomas (NCI-H128 and NCI-H69) which grow as floating aggregates were also maintained as described above. The cells were serially subcultured during the course of the study which was carried out on cell cultures of passage numbers between 15 and 30.

In some experiments cells were preincubated with BNF; cells were placed in culture medium with 50 μM BNF in DMSO for 21 h (21). Cells in the control group were preincubated with the same medium lacking BNF. The concentration of DMSO never exceeded 0.5% v/v.

Prior to use, the cell monolayer was washed twice with Hank's balanced salt solution. In the presence of salt solution, the cells were scraped off at 4°C and transferred to 50-mL centrifuge tubes. Floating aggregates, which did not require scraping, were transferred directly to centrifuge tubes. Following centrifugation at 800 rpm and a further wash in Hank's balanced salt solution, the cells were resuspended in an appropriate volume of Krebs-Henseleit medium, pH 7.4, supplemented with 12.6 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. When incubations were carried out in the presence of NADPH (1 mM), the cells were homogenized prior to incubation.

Incubations. Incubations were carried out in 10-ml Erlenmeyer flasks in a gyratory water bath at 37°C. The incubate contained 3–5 × 104 cells/ml in a final volume of 2 ml. Except for incubations carried out under nitrogen or carbon monoxide/oxygen, all reactions were carried out under an air atmosphere. Nitrogen or carbon monoxide/oxygen (80:20) was bubbled into the buffer for 10 min prior to addition of the cell suspension and NADPH. The flask contents were then sealed with injectable septa and gassed for the entire preincubation and incubation periods. Following a 3-min preincubation, the reaction was started by adding the appropriate amount of radiolabeled IPO in 15 μl of methanol by microsyringe through the septa. The incubate contained 10 μCi of radioactivity in a final concentration range of 0.125–5 mM substrate. Incubations were run for 10 min, except for the time-dependence study, and were terminated by adding 1 ml of 20% trichloroacetic acid. In control mixtures, the cell suspensions were incubated (5 min) in a boiling water bath prior to experimentation.

Assay for Covalently Bound Radioactive IPO Metabolite(s). Following precipitation of the protein with trichloroacetic acid the cells were sonicated to disrupt membranes (this step was not carried out when cells were homogenized prior to incubation). After centrifugation, the precipitates were assayed for covalently bound radioactivity after solvent extraction to remove the nonbound compound(s), as previously described (12, 17). The precipitate was washed with methanol (3-mI aliquots) until no radioactivity could be detected in the supernatant fraction (usually 10 washes were required). The washed precipitates were solubilized in 1 N sodium hydroxide and aliquots were removed for scintillation counting and protein determination.

Counting was carried out after adding 10 ml of Ultrafluor scintillation cocktail (National Diagnostics, Somerville, NJ) using a Packard Tri-Carb 4530 liquid scintillation counter with automatic external standardization. Protein was determined by the Bradford method (22).

Cytotoxicity. Two different assays were used to test the cytotoxicity of IPO to the human lung cancer cell lines. This was necessitated by the fact that the nonsmall cell lines propagated in vitro as monolayer cultures and grew very poorly in suspension or in semisolid medium (i.e., soft agar), whereas the small cell lines grew as floating cell aggregates and thus drug-induced cytotoxicity could be assayed easily by standard agar cloning techniques; therefore, the cytotoxicity of IPO on the two non-small cell lines NCI-H322 and NCI-H358 was carried out by a method described previously by McMahon and Lyte (23), which measures colony forming ability of attached cells. Briefly, cells were plated at 500 cells/60-mm dish in RPMI 1640 supplemented with FBS (10% v/v), glutamine (2 mM), and gentamycin (50 μg/ml, w/v) (control medium). Following a 24-h incubation at 37°C in an atmosphere of 95% air/5% CO2 to allow for attachment, the medium was removed and replaced with medium containing various concentrations of IPO in DMSO. Control medium contained DMSO alone at a final concentration of 0.05%. Cells were incubated in the presence of the drug for 4 days, after which the medium from all plates was replaced with control medium. The cells were reincubated for an additional 5 days (recovery period) and then fixed with methanol and stained with Giemsa. The total number of standard cell colonies greater than 0.5 mm diameter was determined using an Artek Model 880 automatic colony counter (Artek Systems, Farmingdale, NY). Cytotoxicity of IPO on the small cell lines NCI-H69 and NCI-H128 was assessed by the soft agar human tumor clonogenic stem cell assay as described by Hamburger and Salmon (24). After the incubations with and without IPO, the NCI-H69 and NCI-H128 cells were resuspended in control medium and cell aggregates were broken up to single cells, with minimal cell loss, by rapid trituration. The cells were then resuspended in control medium and 0.3% agar at 41°C and plated at a concentration of 5 × 103 cells over 0.5% agar in 60-mm Petri dishes. Dishes were incubated at 37°C for 14 days in 5% CO2/95% air. Plates were examined 24 h after plating to verify that no artifactual cell clusters were present. After 14 days clusters containing more than 20 cells were counted with phase microscopy. All tests were conducted in triplicate.

Statistics. Statistical analysis was carried out using Student's t test for nonpaired samples. A value of P < 0.05 was considered statistically significant.

RESULTS

Production and Covalent Binding of Highly Reactive [3H]IPO Metabolite(s). Both cell lines derived from non-small cell carcinomas (NCI-H322 and NCI-H358) mediated the conversion of IPO to a reactive intermediate, as evidenced by covalent binding of the [3H] label (Table 1). The level of [3H] binding was significantly (P < 0.005) but not strikingly higher for the cell line with Clara cell features (NCI-H322) compared to NCI-H358 (with features of alveolar type II cells). Covalent binding was not detectable in the small cell lines derived NCI-H69 and NCI-H128 (limit of detection, 18 pmol/10 min/mg protein).

Covalent binding of the [3H] label was substrate concentration dependent with both NCI-H322 and NCI-H358 and had significant. For nonpaired samples. A value of P < 0.05 was considered statistically significant.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>pmol/10 min/106 cells</th>
<th>pmol/10 min/mg protein</th>
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<tr>
<td>NCI-H322</td>
<td>46.6 ± 1.08</td>
<td>256.6 ± 6.00</td>
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<tr>
<td>NCI-H358</td>
<td>32.9 ± 0.74*</td>
<td>197.7 ± 4.44*</td>
</tr>
<tr>
<td>NCI-H69</td>
<td>Not detectable</td>
<td>&lt;18</td>
</tr>
<tr>
<td>NCI-H128</td>
<td>Not detectable</td>
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* Significantly different (P < 0.005) from the value for NCI-H322.

Fig. 1. Structure of 4-ipomeanol.
ACTIVATION OF 4-IPOMEANOL BY HUMAN LUNG CANCERS

The $K_m$ value for $^3$H covalent binding for NCI-H322 was approximately 1.5 times lower than that for NCI-H358 (263.3 and 406.0 $\mu$M, respectively).

**Time Course for Production and Covalent Binding of $[\text{H}]$IPO Metabolites.** The *in vitro* activation of $[\text{H}]$IPO by NCI-H322 and NCI-H358, as evidenced by $^3$H covalent binding to cell macromolecules, was linear over 2–10 min (Fig. 3). There was an initial lag phase which was more pronounced with NCI-H322. The reaction had essentially reached a plateau after 10 min with NCI-H358 as compared to 15 min with NCI-H322.

**Modulation of IPO Activation by Known Inducers and Inhibitors of the Monooxygenase Enzyme System.** Covalent binding of $^3$H (to cellular macromolecules) following incubation with tritiated IPO was measured after homogenization of the cells and addition of NADPH (1 mM) and was compared to the covalent binding obtained in intact cells under the same conditions. The presence of exogenous NADPH in the incubate produced a significant increase (25% for NCI-H322; 20% for NCI-H358) in the amount of covalently bound material (Fig. 4). Very low levels of binding were obtained with heat-denatured cells, indicating that the reaction was enzyme mediated; similarly, incubation in the presence of a carbon monoxide-enriched atmosphere or in the presence of an oxygen-free nitrogen atmosphere strongly inhibited the activation of $[\text{H}]$IPO, as shown by the decrease in $^3$H covalent binding (Fig. 4). Attempts to further elucidate the relationship between metabolism and cytotoxicity in these tumor lines using piperonyl butoxide, a known inhibitor of IPO metabolism in other systems (11–13), were unsuccessful; the piperonyl butoxide was itself toxic to all the cultured cell lines under the conditions studied.

The effect of prior treatment with BNF, a known inducer of mixed-function oxidase activity in other *in vitro* systems (21), was measured in the two nonsmall cell lines. The effect of the BNF treatment on the metabolic potential of the cells was monitored initially by changes in 7-ethoxycoumarin O-deethylase activity, which was significantly increased (20-fold for NCI-H322 and 15-fold for NCI-H358). Paralleling the latter enhancement, there were indeed significant increases in the amounts of $[\text{H}]$IPO bound covalently to both NCI-H322 and NCI-H358 cell macromolecules (55 and 30% increase, respectively), although to a far lesser extent than with the 7-ethoxycoumarin O-deethylase (Fig. 4). When BNF-pretreated cells were homogenized prior to incubation with $[\text{H}]$IPO (in the presence of 1 mM NADPH), the increase in $^3$H covalent binding was not significantly different from that obtained using intact cells (data not shown); similarly, in homogenized cells (in the...
presence of NADPH), carbon monoxide inhibited the covalent binding of $^3$H label to the same extent as in intact cells (data not shown).

Toxicity. IPO exerted a dose-dependent cytotoxicity on both NCI-H322 and NCI-H358 as evidenced by the reduction in the number of cell colonies present following treatment (Table 2); moreover, the cytotoxicity of IPO was more extensive with NCI-H322 than with NCI-H358; however, using the same IPO concentrations that gave these pronounced effects on the non-small cell lines, there was no effect on the proliferation of the small cell lung cancer cells in soft agar under the conditions employed (Table 2).

DISCUSSION

The studies described herein indicate that certain human non-small cell lung cancers have metabolic characteristics of normal bronchiolar Clara cells and alveolar type II cells. Following incubation of [H]$^3$HIPO with either NCI-H322 or NCI-H358 lines, there was metabolism-dependent covalent binding of a $^3$H-labeled metabolite to cell macromolecules. Covalent binding was somewhat greater in the NCI-H322 cell line, as was also the relative cytotoxicity. As has been described previously (20), NCI-H322 shows morphological features of Clara cells including the presence of small amounts of smooth endoplasmic reticulum and electron-dense secretion granules, whereas NCI-H358 shows features of the alveolar type II cells and is active in phospholipid synthesis. Under the in vitro growth conditions used, neither metabolic activation nor cytotoxicity of IPO could be detected in the small cell lung cancer lines NCI-H69 and NCI-128. These small cell lines are rich in endocrine activity but lack a characteristic endoplasmic reticulum (20). The covalent binding of $^3$H to cell macromolecules required prior metabolic activation of [H]$^3$HIPO in these cell lines as demonstrated by the use of heat-denatured cells as a control, where no significant covalent binding occurred. The metabolic activation of [H]$^3$HIPO in the non-small cell lung cancer lines required aerobic conditions, was strongly inhibited by a carbon monoxide enriched atmosphere, and was enhanced by exogenous NADPH. Pretreatment of NCI-H322 and NCI-H358 cell lines with BNF resulted in a significant increase in the amount of covalently bound $^3$H label following incubation with [H]$^3$HIPO. Collectively, these results are consistent with the view that the metabolic activation of IPO in these cells is mediated by a monooxygenase system; the characteristics of this system suggest the involvement of some form(s) of a cytochrome P-450 isozyme. The metabolic studies are therefore also consistent with a Clara cell origin for NCI-H332 and an alveolar type II cell origin for NCI-H358. In the non-small cell lines, BNF caused an enhancement of ethoxycoumarin O-deethylase activity by 16- to 20-fold but IPO binding to cellular macromolecules was increased only 1.3- to 1.5-fold. This suggests that a BNF-inducible isozyme(s) of cytochrome P-450 in these cells is not a major form normally involved in the metabolism of IPO. Other inducers of cytochrome P-450 (phenobarbital and dexamethasone) did not produce a detectable induction of any cytochrome P-450 isozymes in these 2 cell lines as estimated by ethoxycoumarin O-deethylase and aldrin epoxi-
dase activities. The functional significance of the presence of a highly specialized cytochrome P-450-related monooxygenase system in bronchiolar Clara cells or in alveolar type II cells is unknown; however, it is interesting to speculate that the unusual propensity of this system for the xenobiotic 4-ipomeanol derives from some key resemblance of IPO to an endogenous substance which may be a "normal" physiological substrate for the lung cell monooxygenase system.

The precise chemical nature of the reactive cytotoxic metabolite of 4-ipomeanol is still unknown; however, recent studies with simpler furan compounds such as 2- and 3-methylfuran indicate that the heterocyclic ring undergoes an initial oxidation, followed at least in the case of the methylfurans, by ring opening to give highly reactive unsaturated aldehydes (e.g., acetylacrolein and methylbutenenedial from 2- and 3-methylfuran, respectively) (25). The reactive metabolite of IPO can be formed and trapped in vitro with lung microsomal systems in the presence of high concentrations of glutathione (reduced form) (26). Although this is a useful alternative approach for the indirect quantitation of reactive IPO metabolites, the resulting reduced glutathione conjugates have proved too unstable for isolation or detailed structural studies. A recent review of the chemistry and potential bioactivation mechanisms for furan compounds is available (27).

Our current experiments suggest a relationship between the ability of tumor cells to metabolically activate IPO and a higher susceptibility to its cytotoxic effects. A positive correlation between metabolic activation and cytotoxicity is suggested herein by the comparisons between the two non-small cell tumor lines (i.e., metabolism and cytotoxicity) and the small cell lines (i.e., no metabolism and minimal cytotoxicity). It must be cautioned, however, that this still represents a wholly inadequate sampling of either of the broad categories termed "non-small cell" or "small cell" lung tumors. An extensive sampling of a broad variety of tumor materials including fresh tumors and primary and early passage cultures will be necessary before any relationship between metabolic potential and enhanced or diminished cytotoxicity can be more clearly defined.

The observation of cytotoxicity of 4-ipomeanol against at least some human nonsmall cell lung cancer lines, especially one of presumed Clara cell origin, is intriguing. In terms of "potency," the effects of IPO against even the more sensitive non-small cell line is not especially remarkable in the present experiments (~1 log cell kill; see Table 2); however, the possible differential cytotoxicity is of greater interest. Clearly, IPO is not a general cytotoxin, as exemplified by its minimal effect on the two particular small cell lung cancer lines tested at extraordinarily high IPO concentrations (10 mM). Several detailed follow-up investigations in other laboratories by different investigators using different assay procedures and/or end points

<table>
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<th>Table 2 Concentration-dependent cytotoxicity of 4-ipomeanol to human lung cancer cell lines</th>
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<td>Non-small cells were incubated with 4-ipomeanol for 4 days. Following a 5-day recovery period, the cell colonies attached to the plastic dishes were fixed with methanol and stained with Giemsa. Results are mean ± SD of the number of colonies with a diameter larger than 0.5 mm/dish, from 3 dishes/dose. Values in parentheses are percentages of the respective control value.</td>
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<td>Concentration (mM)</td>
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3 Unpublished observations.
have subsequently provided qualitative confirmation of the in vitro cytotoxicity of IPO against human nonsmall cell lines; moreover, initial experiments in an in vivo xenograft model using a new proprietary microencapsulation technique have shown evidence of in vivo activity of IPO against NCI-H332. Detailed accounts of these follow-up studies will be presented in separate manuscripts.

The further investigation of the potential utility of 4-ipomeanol or other preferential lung cell toxins as a possible new avenue for experimental therapeutics of lung cancer seems clearly warranted. This view was initially forwarded for IPO in 1977 (11), but further studies were complicated by the lack of suitable tumor model systems for study; indeed, recent evaluations of IPO in all of the NCI in vivo tumor model systems that were available routinely for initial and/or follow-up testing during 1975–1984 were, not surprisingly, uniformly negative.

The relative proportion of human bronchogenic lung cancers originating from Clara cells is unknown, although documented cases with “classic” Clara cell features are relatively rare (28); however, routine histopathology does not allow for an unequivocal identification of Clara cell features in tumors, which may in turn explain why such tumors are not diagnosed more often. Moreover, given the recent appreciation of the “stem-cell” role of normal Clara cells plus the presence of an exceedingly active and specialized metabolic system capable of activation of toxins in these cells it would not be surprising if a much larger proportion of human lung cancers than heretofore imagined are of Clara cell origin. This speculation is, of course, based primarily upon experimental studies largely performed in rodents; there clearly are some morphological differences among pulmonary Clara cells of different animal species including humans (29); nevertheless, the results of the experiments reported herein on human materials suggest that the conclusions and concepts derived initially from animal experiments are most likely also valid for humans. Further insight in this regard will require more detailed characterization of xenobiotic metabolizing systems in normal human lung tissue and lung cells as well as in a representative spectrum of human lung cancer subtypes. IPO should serve as a useful probe for such experiments because it offers a convenient way to measure with a high degree of sensitivity and specificity a metabolic pathway implicated closely with the key target lung cells and tumors derived therefrom; indeed, it may be of considerable interest to survey a large number and a broad representation of human tumors for the presence of an IPO-metabolizing system; in this way it may be possible to more accurately define a true subset of bronchogenic and/or bronchioloalveolar lung cancer patients whose tumors are likely to be derived from normal cell populations with IPO-metabolizing capacity. Such a “biochemical tumor screen” might also be useful for defining a lung cancer patient population optimal for clinical testing of 4-ipomeanol.

Finally, the cell lines (especially NCI-H332) and NCI-H358 described herein may be useful for incorporation into in vitro screens for new agents potentially active against specific lung cancers. The cells might also be useful in test systems designed to detect agents potentially toxic to normal lung cell types. The possible usefulness of such cell lines for these purposes is further suggested by our observations that the morphology, the biochemical characteristics assessed here (response to BNF induction and ethoxycoumarin O-deethylase activity), and the response to IPO did not change substantially after repeated passaging when compared to early passage cells.

The National Cancer Institute has recently begun the development and implementation of a new “disease-oriented” primary drug screening program. This program is intended to provide an alternative to the existing “compound-oriented” drug screen which has relied predominantly upon the mouse leukemia P388 in vivo prescreen (e.g., see Refs. 30–33). The new screens will incorporate initial testing of compounds in vitro against broadly representative panels of major human cancer types for which few if any active drugs have been discovered. The initial emphasis in the program is on lung cancer both because it is the commonest lethal cancer occurring in the United States and many other countries worldwide, and also because, especially in the case of the non-small cell category which comprises the majority of lung cancers, there are no active drugs nor other effective treatments. Current projections (34) suggest an expected incidence of approximately 150,000 new cases of lung cancer in 1986 and approximately 130,000 or more deaths due to the disease. Of these cases, generally 80% or more may be expected to be of the non-small cell varieties (28, 34).

Because of the urgent need for new drug candidates with any kind of potentially exploitable activity against lung cancer and because of the rationale discussed herein 4-ipomeanol has been selected recently by NCI for consideration for clinical trials emphasizing lung cancer. The experimental studies described herein suggest that such an approach is warranted; moreover, IPO may provide an initial prototype drug with which to begin to explore the usefulness of the new NCI screening program. IPO is clearly one type of agent that the new screens would be likely to detect but which would clearly have been missed by the prior and other existing screens that have relied predominantly upon the murine leukemias for initial detection.

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