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Metabolism of Benzo(*a*)pyrene and 1-Naphthol in Cultured Human Tumorous and Nontumorous Colon[1]

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ABSTRACT

The oxidative metabolism of benzo(*a*)pyrene and the conjugative metabolism of 1-naphthol by explant cultures of normal human colon and colonic tumor tissue, obtained at surgery, have been studied. After 24 hr in culture, the explants were exposed to either [1-^{14}C]-1-naphthol (20 to 100 μM) or [^{3}H]-benzo(*a*)pyrene (1.5 μM) for a further 1.5 to 24 hr. Both normal-appearing tissue and tumor tissue metabolized benzo(*a*)pyrene to a wide variety of organic solvent-soluble metabolites, including monohydroxybenzo(*a*)pyrenes, dihydrodiols, and tetrols. 1-Naphthol was metabolized by cultured human colonic mucosa and tumor tissue to both its glucuronic acid and sulfate ester conjugates. In the normal tissues, with naphthol (20 μM), sulfate ester conjugation predominated. However, with the tumor tissue, sulfate ester conjugation decreased; thus, the percentage of glucuronic acid conjugates, expressed as a percentage of total metabolites formed, was increased significantly compared to normal tissue. The relationship, if any, of these changes to neoplastic transformation is unclear. The technique of explant culture described in this study may be of use for the study of other facets of the pathobiology of solid tumors.

INTRODUCTION

Epidemiological studies have implicated dietary factors as being of major importance in the etiology of colon cancer (26). The prognosis for patients with this disease is generally poor, and the results with chemotherapy have been very disappointing (26). As one approach to this problem, we are studying biochemical differences between normal and tumor tissues from patients with colon cancer, with particular reference to xenobiotic metabolizing enzymes.

Xenobiotic metabolism is generally considered to take place primarily, although not exclusively, in the liver in 2 phases, *i.e.*, Phase I and Phase II reactions (27). The majority of studies on the xenobiotic metabolizing enzymes in normal and tumor tissues have concentrated on Phase I oxidative reactions, most commonly in rodent hepatomas of different growth rates (1, 22, 25, 29). In general, these studies demonstrate the presence of cytochrome P-450 mixed-function oxidase activities in such tumors, although such activities are generally much lower than those from corresponding controls and do not appear to correlate with the growth rate of the hepatoma (25, 29). A recent study has shown that aryl hydrocarbon hydroxylase is also significantly lower in homogenates from tumors of patients with lung cancer than from corresponding "normal" lung tissue from the same patients (24). Various rodent hepatomas have been demonstrated to possess increases in UDP-glucuronosyltransferase activities (17, 28). Using a Reuber H-35 hepatoma, Gessner (15) reported that the hepatoma possessed a higher UDP-glucuronosyltransferase activity but a very low sulfotransferase activity compared with activities in liver from either controls or tumor-bearing animals. Dao and Libby (9) noted that human mammary neoplasms had a variable pattern of steroid-sulfating activity which differed from those of either normal breast tissue or normal liver. Short-term organ cultures of normal human peripheral lung metabolize 1-naphthol primarily to its sulfate ester conjugate (19), whereas tumor tissue from the same patients, in particular those with squamous cell carcinomas, forms almost exclusively the glucuronic acid conjugate (7, 20).

From the above studies, it appeared that there may be significant differences in conjugation pathways utilized by certain normal-appearing and tumor tissues. In order to test and extend this hypothesis, the present study was designed to investigate both the oxidative and conjugating ability of normal human colonic mucosa and colonic tumor tissue from the same patients.

MATERIALS AND METHODS

Specimens. Normal-appearing human colonic tissue and tumor tissue were obtained at the time of surgery (Patient C5, 56-year-old male; Patient C8, 66-year-old male; Patient C10, 63-year-old male; Patient 220C, 44-year-old male; Patient 220E, 60-year-old female; Patient 220H, 66-year-old female; Patient 220I, 71-year-old male; Patient 221B, 62-year-old female). No patient had received prior treatment with either radiotherapy or cancer chemotherapeutic agents. Explants of colon were cultured in a chemically defined medium (CMRL-1066) essentially as described by Autrup (3, 4). The major modification was that the colon was cultured on gelatin sponge (Gelfoam; The Upjohn Co.) in order to compare the results with those obtained with the tumor tissues. Two small explants of tumor tissue (approximately 2 × 2 × 1 mm) were placed on a piece of gelatin sponge and gassed with 95% O_2/5% CO_2. The explants were then cultured for 24 hr, after which the medium was replaced by one containing either [^{3}H]BP[4] (40 to 66 Ci/mmol; 1.5 μM) or [1-^{14}C]-1-naphthol (19.4 mCi/mmol; 20 to 100 μM), both radiolabeled substrates obtained from Amersham/Searle, Arlington Heights, Ill.

Analysis of BP Metabolites by High-Performance Liquid Chromatography. After 24 hr culture with [^{3}H]BP, the medium was removed and extracted twice with ethyl acetate/acetone (2/1, v/v). Unmetabolized BP was removed by chromatography on a SEP-PACK column, and BP metabolites were analyzed as described previously (5).

Metabolism of [1-^{14}C]-1-Naphthol. After 24 hr culture with [1-^{14}C]-1-naphthol, the medium was removed and stored at −20° until further

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[4] The abbreviation used is: BP, benzo(*a*)pyrene.

analysis. The conjugates in the media were analyzed by thin-layer chromatography essentially as described previously (19). Controls were obtained by culturing media containing [^{14}C]naphthol for 24 hr in the absence of tissue, and the media were removed and analyzed as described previously (19).

Histology. Samples of normal-appearing and tumor tissue from before, during, and after culture were fixed in formalin/glutaraldehyde (18) and examined by light microscopy after the sections were stained with either hematoxylin and eosin or Alcian blue/periodic acid-Schiff.

RESULTS

Morphology of the Colonic Tissues. The culture conditions were satisfactory for the maintenance of both nontumorous and tumorous colonic tissue when cultured for 24 or 48 hr. Microscopic examination of the colon cancers revealed 2 histological types: (*a*) well-differentiated adenocarcinoma (Patients 220C, 220E, 220H, C5, C8, and C10); and (*b*) poorly differentiated adenocarcinoma (Patients 220I and 221B).

Metabolism of BP. BP was metabolized by short-term organ cultures of human colon and tumor tissue to a wide variety of different organic solvent-soluble metabolites including dihydrodiols, phenols, quinones, triols, and tetrol (Table 1). A large interindividual variation was noted in the percentage of different metabolites formed by both the normal-appearing and tumor tissues (Table 1). No significant difference was observed between these tissues, although the total percentage of tetrols was somewhat increased in tumor tissue from all 4 cases. Greater variation occurred between individuals than between normal and tumor tissue obtained from the same individual as indicated by the formation of 2 of the major metabolites, *i.e.*, 9,10-dihydro-9,10-dihydroxybenzo(*a*)pyrene and 7,8-dihydro-7,8-dihydroxybenzo(*a*)pyrene.

Metabolism of 1-Naphthol. Short-term organ cultures of both human colon and tumor tissue metabolized 1-naphthol to both its glucuronic acid and sulfate ester conjugates (Table 2). The overall metabolism of 1-naphthol in the tumor tissue was decreased compared to normal colon. With a naphthol concentration of 20 μM, macroscopically normal colon formed significantly more 1-naphthyl sulfate than 1-naphthyl-β-D-glucuronide. When the substrate concentration was increased to 100 μM, the percentage of substrate conjugated with sulfate decreased, whereas the percentage conjugated with UDP-glucuronic acid increased (Table 2).

When colonic tumor tissue from the same patients was cultured with 1-naphthol (20 μM), a marked decrease in the percentage of sulfate conjugates was observed (Table 2). The percentage of glucuronic acid conjugates, expressed as a percentage of total metabolites formed, was increased significantly compared to normal tissue ($p < 0.05$) (Table 2). Similar results were also obtained when the normal and tumor tissue were cultured with 1-naphthol (20 μM) for only 90 min (results not shown). However, for ease of detection and quantification of results, the experiments were generally carried out for 24 hr.

DISCUSSION

We have described a method, using similar experimental conditions, for the maintenance of normal-appearing colonic tissue and tumor tissue, from the same patients for periods up to at least 48 hr. Several other systems have been described for the culture of normal human colon (3, 4) and colonic tumor tissue (16), but generally the conditions have been very different, thus making comparative studies particularly difficult. In addition to their use for studying drug metabolism, such systems may also be of value in studying other biochemical differences between normal and tumor tissues.

A large interindividual variation was observed in the nature of the organic solvent-soluble metabolites formed from BP by cultured colonic mucosa (Table 1) in agreement with the observations of Autrup *et al.* (4). A similar variation was also observed between the different tumors (Table 1). However, relatively little variation was observed between normal-appearing and tumor tissue from any individual patient. Both normal-appearing and tumor tissue formed several metabolites including monohydroxybenzo(*a*)pyrenes [3-hydroxybenzo(*a*)pyrene and 9-hydroxybenzo(*a*)pyrene], dihydrodiols [in particular 7,8-dihydro-7,8-dihydroxybenzo(*a*)pyrene and 9,10-dihydro-9,10-dihydroxybenzo(*a*)pyrene], and tetrols. A relatively large amount of uncharacterized relatively polar material was eluted

Table 1

Organic solvent-soluble BP metabolites formed by cultured normal-appearing and tumor tissue of human colon

Explant cultures were incubated at 1.5 μM [^{3}H]BP (40 to 66.3 Ci/mmol) for 24 hr at 37° before medium was collected and analyzed by high-performance liquid chromatography for BP metabolites. Generally, 2 to 6 dishes of normal-appearing colon or tumor tissue from each patient were pooled, and the media were analyzed for metabolites.

Metabolites	Typical retention time (min)	% of total metabolites							
		220C		220E		220H		221B	
		N[a]	T	N	T	N	T	N	T
Unidentified	5.0–8.0	26.7	7.9	56.5	38.9	34.4	47.1	8.1	8.8
(7,10/8,9)-Tetrol	14	1.1	1.8	0.1	0.2	0.6	3.0	0.3	1.8
(7,9/8,10)-Tetrol	16.5	0.6	1.0	0.1	0.1	0.5	0.5	0.8	4.4
(7/8,9,10)-Tetrol	18	0.3	0.3	0.3	1.0	0.5	0.1	0.1	0.3
9,10-Diol + (7/8,9)-triol	20	30.3	39.6	7.4	6.6	22.7	24.5	52.3	35.2
(7,9,10/8)-Tetrol	21.5	0.1	0.1	0.1	0.5	1.9	0.5	0.4	2.6
4,5-Diol	30.5	0.5	1.7	0.6	0.4	2.0	0.2	0.1	0.1
7,8-Diol	32.5	16.4	31.0	6.5	7.5	9.8	9.7	28.4	28.1
9-OH-BP	44.0	0.5	1.1	3.8	5.0	3.6	1.5	0.5	2.0
3-OH-BP	46.0	10.9	6.3	9.7	14.7	5.5	5.5	1.4	3.1
Quinones	49.0–53.0	1.1	1.7	2.5	7.5	4.9	3.0	3.0	1.1
Unidentified	Various	11.5	7.5	12.5	17.7	13.6	4.5	4.7	12.5

[a] N, normal-appearing tissue; T, tumor tissue; 9,10-diol, 9,10-dihydro-9,10-dihydroxybenzo(*a*)pyrene; 4,5-diol, 4,5-dihydro-4,5-dihydroxybenzo(*a*)pyrene; 7,8-diol, 7,8-dihydro-7,8-dihydroxybenzo(*a*)pyrene; 9-OH-BP, 9-hydroxybenzo(*a*)pyrene; 3-OH-BP, 3-hydroxybenzo(*a*)pyrene.

Table 2

Metabolism of 1-naphthol by cultured normal-appearing colon and tumor tissue

Short-term organ cultures of normal-appearing human colon or tumor tissue were cultured on gelatin sponge in 2.5 ml of CMRL-1066 medium as described. After 24 hr, the culture medium was replaced by one containing [1-^{14}C]-1-naphthol (20 or 100 μM) and cultured for a further 24 hr. Aliquots of the media were analyzed for 1-naphthol conjugates by thin-layer chromatography. The results are expressed as the mean values from 1 to 6 dishes, each dish containing 2 explants.

		% of total radioactivity in the medium as conjugates in				Glucuronic acid conjugate as % of total metabolites	
		Colon		Tumor			
Patient	Naphthol (μM)	Sulfate	Glucuronide	Sulfate	Glucuronide	Normal	Tumor
220C	20	39.7	5.4	23.0	5.3	12.0	29.0
	100	26.9	15.9				
220E	20	53.5	3.3	23.0	21.7	5.8	48.5
220F	20	18.0	0.06	[a, b]	[a]	[a]	[a]
	100	3.2	0.05	[a]	[a]	[a]	[a]
220H	20	37.8	6.0	6.6	10.7	13.7	61.8
	100	24.0	18.7	[b]	[b]	[b]	[b]
220I	20	39.8	6.0	7.1	11.2	13.1	61.2
	100	24.6	10.5	3.4	11.0	29.9	76.3
C5	20	20.1	2.6	3.0	19.1	11.5	86.4
C8	20	48.5	5.8	1.7	15.1	10.7	89.9
C10	20	39.4	4.7	12.0	28.2	10.7	70.1

[a] This patient had Crohn's disease.
[b] Not determined because of insufficient tissue.

between 5 and 8 min (Table 1). This early eluting material may be polyhydroxylated metabolites or sulfate conjugates of monohydroxybenzo(*a*)pyrenes which have been shown to be organic solvent soluble (8).

1-Naphthol (20 μM) was metabolized by normal-appearing human colon predominantly to its sulfate ester conjugate (Table 2). This is in agreement with the results of Autrup (2), who showed that, when BP (1.5 μM) was incubated with normal human colon, sulfate ester and glutathione conjugates were the major conjugates, with only small amounts of glucuronic acid conjugates being formed. In the present study, when the naphthol concentration was increased to 100 μM, the percentage of sulfate conjugate decreased while the glucuronide conjugate increased. The relative extent of conjugation of a phenol with either glucuronic acid or sulfate is dependent upon the species, the tissue, and the structure and concentration of the substrate (6, 21). Sulfation is a readily saturable process most probably because of a limited availability of sulfur-containing amino acids required for the synthesis of the sulfate donor, adenosine 3′-phosphate 5′-phosphosulfate. Thus, as the concentration of naphthol was increased, a decrease in the amount of sulfate conjugate was observed accompanied by an increase in glucuronic acid conjugation. The latter process requires UDP-glucuronic acid which presumably can be made readily available from carbohydrate precursors in the cultured colon or the medium.

When tumor tissue was incubated with naphthol (20 μM), a much higher percentage of the total metabolites formed was due to glucuronic acid than to sulfate ester conjugates (Table 2). This is in agreement with our results with short-term cultures of human lung and tumor tissue, when the lung forms almost exclusively 1-naphthyl sulfate but the tumor, in particular from squamous cell carcinomas, forms almost entirely the glucuronic acid conjugate (7, 20). The differences in conjugation observed in the present study may be due to a variety of reasons, including alterations in aryl sulfatase and β-glucuronidase activities. Alterations of these enzymic activities have been reported in a variety of different human tumors including colorectal carcinoma (12, 14, 23). A study of both conjugating and deconjugating enzymes, in tissues from the same patient, is required to clarify the possibility.

Alterations in the enzyme protein(s) or in the generation of the appropriate cofactors in the tumor tissue may also explain the differences in conjugation. This is further complicated by the multiplicity of both UDP-glucuronosyltransferase (11) and sulfotransferase (10), and it would be interesting to know if similar changes in conjugation also occurred with other substrates. The significance of these alterations in conjugation is not clear, although it may be related to changes in mucus secretion. A large proportion of colonic mucins from normal human colon consists of sulfomucins, whereas in colonic tumor tissue a marked decrease or absence of sulfomucins is accompanied by an increase in sialomucins (13). While a decreased availability of adenosine 3′-phosphate 5′-phosphosulfate in the tumor tissue would be consistent with both these alterations, other possibilities, such as a decrease in one or more sulfotransferases, must also be considered.

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