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ABSORPTION OF 2-ACETYLAMINOFLUORENE IN THE GUINEA-PIG COLON

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Summary

Absorption of the known chemical carcinogen, 2-acetylaminofluorene has been measured in the colon of guinea pigs. Unidirectional influx across the luminal cell membrane was determined in vitro, and transmural absorption across colonic mucosa was evaluated in vivo.

The kinetics of unidirectional influx into colon in vitro do not indicate that absorption proceeds by simple diffusion. The observed saturable uptake is indicative of binding of 2-acetylaminofluorene to a cellular component. With 2-acetylaminofluorene present in the lumen in vivo at an initial concentration of 3.5 μM, the rate of absorption decreases over a 20 min period, which also indicates some form of specific interaction between 2-acetylaminofluorene and the intestinal mucosa.

We have evaluated the hypothesis that surfactants and a bile salt act as cocarcinogens by increasing the rate of intestinal absorption of 2-acetylaminofluorene. The results lend no support to this possibility.

Introduction

Considerable progress has been made in identifying the specific mechanisms of intestinal transport of many nutrients. The characteristics of absorption and secretion of several substances foreign to the body have also been studied [1—4]. Because some of the xenobiotics are carcinogenic, the rate and mechanism of their transport by the gastrointestinal tract are of considerable interest, although these processes are incompletely understood. The presence of a variety of substances in the intestinal lumen increases the toxic effect of certain carcinogens by unknown mechanisms. Some of these cocarcinogens (e.g bile

salts) increase permeability properties of gastrointestinal mucosa to a wide variety of substances [5,6] perhaps by dissolving phospholipid [7] or protein [8] from the lining epithelial cells. Thus, the possibility may be considered that absorption of carcinogens is increased in the presence of bile salts.

In the present study, absorption of a known chemical carcinogen, 2-acetylaminofluorene, has been investigated by standard techniques in vivo and in vitro in the mammalian colon. Guinea pigs were used because their livers contain little of the enzymic activity which catalyzes N-hydroxylation of 2-acetylaminofluorene [9]; interpretation of studies in vivo would be made more difficult if metabolic conversion of the carcinogen occurred. The possibility was tested that bile salts and surfactants act as cocarcinogens by increasing the rate of absorption of 2-acetylaminofluorene. The kinetics of 2-acetylaminofluorene absorption by guinea-pig colon are found to be inconsistent with a process of simple diffusion and suggest, instead, that binding of 2-acetylaminofluorene to some intestinal cellular component may take place during absorption. Additional studies to determine if 2-acetylaminofluorene is secreted by the colon are indicated.

Materials and Methods

In vitro technique. Guinea pigs (400—500 g) were killed by intraperitoneal injection of pentobarbital. An abdominal incision was made and colon segments were taken just distal to the ileocecal valve. The segments were opened and washed in Ringer (NaCl, 142 mM; $KHCO_3$, 10 mM; K_2HPO_4, 1.2 mM; $CaCl_2$, 1.2 mM; $MgCl_2$, 1.2 mM, pH 7.2; pyruvate, 5 mM). The tissue was mounted, luminal surface up, in a lucite chamber which consisted of six isolated perfusion ports and was similar to the original design of Schultz et al. [10]. In each port, 0.265 cm^2 of mucosal surface was exposed to 37°C Ringer, which was stirred by a fine stream of humidified O_2. The serosal surface of the tissue rested on moistened filter paper and was not exposed to the solution bathing the mucosal surface. The tissue was preincubated in Ringer for 5—10 min.

Preincubation solution was withdrawn from each port individually, and the appropriate test solution with ^{14}C-labeled 2-acetylaminofluorene and [^3H]-inulin was injected into the port. This solution bathed the mucosal surface for 2, 5 or 10 min and was then removed; the port was flushed briefly with cold (4°C) Ringer. The time interval from injection of the test solution to injection of the cold wash was taken as the duration of exposure of the mucosal surface to the isotope. The cold wash served to terminate the influx of isotope both by diluting the test solution remaining in the port and by cooling the tissue. The exposed area of tissue was cut out with a steel punch, washed briefly in cold Ringer, blotted, and extracted in 0.1 M HNO_3 for 18—24 h. Aliquots of tissue extract and test solution were assayed for ^3H and ^{14}C activity by liquid scintillation spectrometry. Uptake of 2-[^{14}C]acetylaminofluorene by the tissue was calculated after correction for the inulin space. Previous studies have shown that inulin does not enter the intestinal cells to a significant extent. Thus, inulin space is a measure of the volume of adherent test solution which was not removed by the cold wash. The results of these experiments provide a reliable

measure of unidirectional influx, provided that the ^{14}C-labeled products of any 2-acetylaminofluorene metabolites are retained within the tissue for the duration of the influx. The method has previously provided reliable information about the absorption of several nutrients and their biologically inert analogues across the luminal border of intestinal cells [11—14]. Uptake of 2-acetylaminofluorene was determined over the mucosal 2-acetylaminofluorene concentration range of 0.015—1 mM by adding appropriate amounts of unlabeled 2-acetylaminofluorene (Aldrich Chemical, Milwaukee, WI) to 2-[^{14}C]acetylaminofluorene (15 μM, New England Nuclear). 2-Acetylaminofluorene was found to be soluble over the concentration range used.

In vivo techniques. Guinea pigs were anesthetized using Penthrane (methoxyfluorene) combined with 1.5 l of oxygen/min. A midline abdominal incision was made and a colon segment was isolated using 1-0 silk ties. Care was taken not to interrupt the blood supply to the isolated tissue. In paired experiments, the control and experimental loops in each animal were adjacent to each other. A small incision was made into the intestinal loop at its most distal point and the loop was washed thoroughly. A 7 cm length of silastic tubing fitted with a luer tip stub adapter was secured into the loop with 1-0 silk ties, and the animal was closed. Ringer was injected into the loop via the silastic tubing.

The preincubation solution for control animals was 1 ml of Ringer. The preincubation solution used in the experimental group of animals was Ringer with one of the following potential cocarcinogens included: polyoxyethylene sorbitan monooleate (surfactant Tween 80, 1%), sorbitan monolaurate (Span 20, 1%) [15,16] or taurodeoxycholate (10 mM) [17,18].

Following a 4 h preincubation period, during which time the animals regained consciousness and became mobile, the loop was washed thoroughly with Ringer to remove the preincubation solution. Test solution consisting of 1 ml of Ringer with 3.5 μM 2-[^{14}C]acetylaminofluorene and tracer quantities of a slowly absorbed marker, [^{3}H]mannitol, was injected into the loop via the silastic tubing. Samples were taken from loops at 1, 10 and 20 min. Results are expressed as means ± S.E.

Results

Uptake of 2-acetylaminofluorene across the luminal cell membrane of colon was investigated at influx periods of 2, 5 and 10 min. The results show (Fig. 1) that uptake is linear with time for at least 5 min. The data extrapolate close to the origin, indicating that no significant amount of the tracer either binds nonspecifically to the tissue or is lost during washings of the tissue. These studies indicate that a reliable measure of 2-acetylaminofluorene influx across the luminal cell membrane is obtained; subsequent influx experiments were performed for 2-min periods.

To aid in determining the mechanism by which 2-acetylaminofluorene is absorbed in guinea-pig colon, influx was determined over a mucosal 2-acetylaminofluorene concentration range of 0.15—1 mM. The results (Fig. 2) show that uptake is not a linear function of the mucosal concentration, but that saturation kinetics are indicated. A plot of 2-acetylaminofluorene concentration/influx against concentration [19] confirms the apparent saturable character

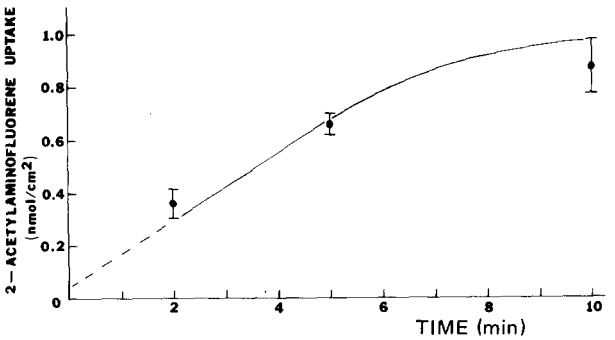

Fig. 1. Uptake of 2-acetylaminofluorene across the mucosal border of guinea pig colon as a function of exposure time of 0.015 mM 2-[^{14}C]acetylaminofluorene. Each bracket is mean ± S.E. of eight influx determinations. Initial slope represents 2-acetylaminofluorene influx of 11 nmol/cm^2 per h.

of influx (Fig. 3). The best-fit straight line indicates that 2-acetylaminofluorene influx across the luminal border of colon can be described by a model for carrier-mediated transport with a calculated K_m of 0.25 mM and a V of 0.28 μmol/cm^2 per h.

Disappearance of 2-acetylaminofluorene from loops of colon in vivo was measured with the carcinogen present in Ringer at an initial concentration of 3.5 μM. In 20 min no significant net fluid movement across the epithelium took place as indicated by constancy of [^{3}H]mannitol activity. In each of six animals investigated, 2-acetylaminofluorene absorption was more rapid between 0 and 10 min than between 10 and 20 min, as evidenced by departure of the data from the dashed line in a semilogarithmic plot of percent 2-acetylaminofluorene remaining versus time (Fig. 4). The possibility may be considered that numerous intracellular binding sites exist which become occupied

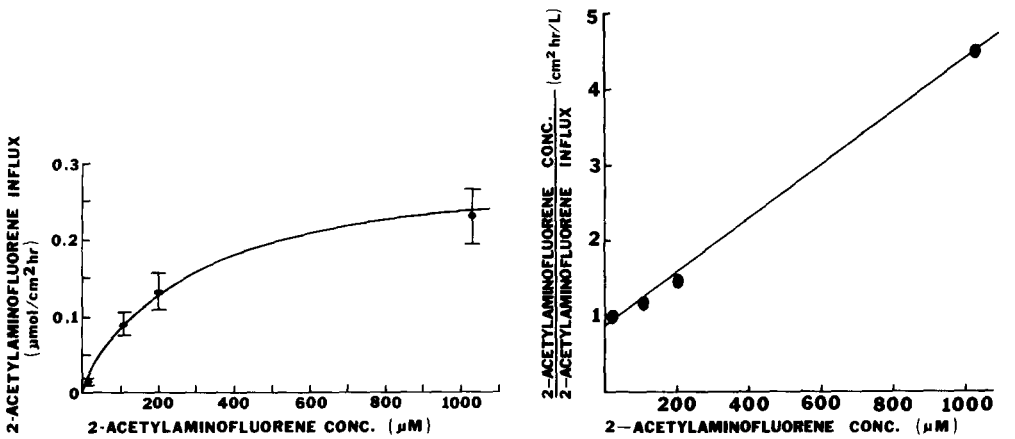

Fig. 2. Influx of 2-acetylaminofluorene into guinea-pig colon from mucosal 2-acetylaminofluorene concentrations of 0.015—1 mM. Each bracket is the mean ± S.E. of six influx determinations.

Fig. 3. Plot of 2-acetylaminofluorene concentration/2-acetylaminofluorene influx vs. 2-acetylaminofluorene concentration. Values were derived from Fig. 2.

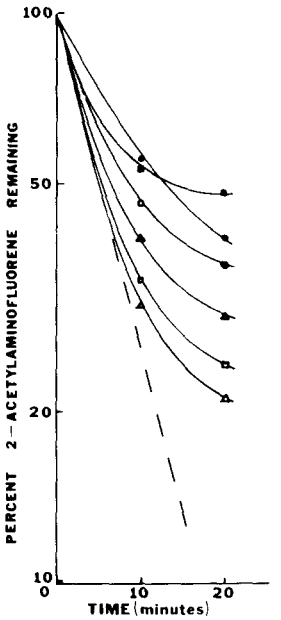

Fig. 4. Disappearance of 2-acetylaminofluorene from a loop of colon in each of six guinea pigs. Note semi-logarithmic plot. ------, rate of disappearance due to simple diffusion with a half-time of 5 min. The data points obtained at one minute (not shown) all had values of approximately 90%.

by 2-acetylaminofluorene. When these binding sites are occupied, the rate of 2-acetylaminofluorene disappearance from the lumen would decrease. This possibility is not given experimental support, however, because in the intestinal loop of these six animals the 2-[^{14}C]acetylaminofluorene extracted from the mucosa at the end of 20 min was only 3.4 ± 0.7% of that lost from the intestinal lumen. Thus, most of the 2-acetylaminofluorene absorbed reached the systemic circulation.

Because surfactants are known to act as cocarcinogens under certain circumstances, the possibility was considered that they have this effect by increasing intestinal absorption of 2-acetylaminofluorene. The short term effect of surfactants was determined by preincubating tissue samples in the influx chambers at 37°C for 15 min either in Ringer (control) or Ringer with 1% Span 20 or Tween 80 (experimental). Neither surfactant had a significant effect on 2-acetylaminofluorene influx (Table I).

The effect of a 4 h exposure of colon to surfactant was determined in vivo by including Tween 80 (1%) in the Ringer of experimental loops during the preincubation period. The control and experimental loops were washed thoroughly to remove the preincubation solution, and test solution was injected into each loop. In five animals the half-time of 2-acetylaminofluorene disappearance from control loops (7.9 ± 0.6 min) was not significantly different from experimental loops (8.4 ± 1.0 min); i.e. the surfactant had no effect on the rate of carcinogen absorption.

Because bile salts are known to affect the permeability properties of gastrointestinal epithelia [5,7], and act as cocarcinogens [17,18], the possibility was

TABLE I

2-ACETYLAMINOFLUORENE INFLUX FOLLOWING TISSUE PREINCUBATION WITH A SURFACTANT OR BILE SALT

Tissue was preincubated for 15 min in vitro in the appropriate solution. Values are the mean ± S.E.; 2-acetylaminofluorene present at 0.015 mM. *N*, the number of influx determinations. n.s., not statistically different.

Preincubation solution	Influx ($nmol/cm^2$ per h)	*N*	*P*
Ringer	16.4 ± 2.4	12	n.s.
Ringer with Span 20 *	15.8 ± 2.8	12	
Ringer	17.2 ± 4.6	12	n.s.
Ringer with Tween 80 *	13.4 ± 4.0	12	
Ringer	25.3 ± 2.6	12	<0.001
Ringer with taurodeoxycholic acid **	9.8 ± 0.4	12	

* Surfactants were present at 1% in the preincubation solution.
** Taurodeoxycholic acid present at 10 mM in the preincubation solution.

considered that taurodeoxycholic acid increases absorption of 2-acetylaminofluorene in the colon. A short-term effect of taurodeoxycholic acid was evaluated in vitro by preincubating colonic mucosa in Ringer (control) or Ringer with 10 mM taurodeoxycholic acid. Taurodeoxycholic acid significantly decreased the influx rate (Table I).

The effect of a 4 h exposure of colon to bile acid was determined in vivo by including taurodeoxycholic acid (10 mM) in the 1 ml of Ringer in experimental loops during the preincubation period. The control and experimental loops were washed thoroughly to remove the preincubation solution, and test solution was injected into each loop. The half-times of 2-acetylaminofluorene disappearance from the paired control (6.8 ± 1.9 min) and experimental (7.5 ± 1.4 min) loops of five guinea pigs were not significantly different.

Discussion

Little information is available about the specific routes by which chemical carcinogens enter the body from the environment. Although it is evident that orally consumed carcinogens are absorbed somewhere along the gastrointestinal tract, the primary site and the mechanism of absorption have not been identified in most cases of interest. Present results in vivo and in vitro suggest that the known carcinogen 2-acetylaminofluorene is rapidly absorbed across the luminal border of guinea-pig colon. Absorption into the mucosa in vitro demonstrates saturation kinetics which are not characteristic of a process of simple diffusion. The time-dependent absorption in vivo also indicates that some form of interaction between 2-acetylaminofluorene and the mucosa takes place. The kinetics in vitro are consistent with a facilitated or active transport process similar to that previously demonstrated to exist for many nutrients [11] and xenobiotics [1—4] in small intestinal mucosa. The results in vivo do not support this hypothesis, however, because the rate of such absorption would not be expected to decrease with time unless considerable accumulation within the mucosa occurred. Accumulation of 2-acetylaminofluorene in the

mucosa was seen to be much smaller than the loss of 2-acetylaminofluorene from the intestinal lumen. The results of both the in vitro and in vivo studies might be explained if the colonic mucosa has a secretory mechanism for 2-acetylaminofluorene. For instance, transport across the luminal cell membrane in the direction opposite the secretory process could show saturation kinetics if 2-acetylaminofluorene interacts with a brush border transport mechanism (carrier) during entry. Absorption in vivo would gradually slow as the concentration gradient between lumen and plasma declined. Secretory processes for other xenobiotics are known to occur in mammalian intestine [20], but it is not known if the intestine functions as an excretory organ for chemical carcinogens. Alternatively, the rate of entry of 2-acetylaminofluorene might be regulated by the intracellular concentration of 2-acetylaminofluorene (i.e. transinhibition). This possibility could also involve the specific recognition of 2-acetylaminofluorene by some membrane component. A more complete understanding of the role of intestine in absorption and secretion of chemical carcinogens might lead to methods of altering the rates of these processes, thereby affecting the carcinogenic potential of some substances which are orally consumed.

The present study provides no support for the hypothesis that surfactants (Span 20, Tween 80) or a bile salt (taurodeoxycholic acid) could act as cocarcinogens by virtue of increasing intestinal absorption of 2-acetylaminofluorene. It is possible, of course, that the use of higher concentrations of detergent and of the bile acid, or of longer periods of tissue exposure to the potential cocarcinogens could have led to a different conclusion. The decrease in 2-acetylaminofluorene absorption following tissue exposure to taurodeoxycholic acid might be due to an effect of the bile salt on a membrane-bound carrier which participates in transmucosal absorption of 2-acetylaminofluorene under our experimental conditions. The reason for a difference in effect when taurodeoxycholic acid is applied in vitro (Table I) versus in vivo (page 00) is not clear.

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