Insulin Promotion of Colon Tumors in Rats[1]

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Abstract

McKeown-Eyssen and Giovannucci have proposed a mechanism for colon carcinogenesis based on the similarity of the risk factors for colorectal cancer and non-insulin-dependent diabetes. They note that diets high in fat and energy and low in complex carbohydrates and a sedentary lifestyle lead to insulin resistance and hyperinsulinemia and propose that the hyperinsulinemia promotes colon carcinogenesis. In this study, we directly tested for a promoting effect of insulin on colon carcinogenesis in F344 rats. After azoxymethane initiation and injections of insulin given 5 times/week for 17 weeks, the fraction of rats with colon tumors was greater in rats receiving insulin than in rats receiving saline (79 *versus* 50%, respectively; $P < 0.05$ for tumors with maximum diameters ≥ 2 mm), and the average number of tumors/rat was also greater (2.00 *versus* 0.73; $P < 0.001$). There was no effect on body weight. Our results demonstrate that insulin is a colon tumor promoter in this rat model and support the proposed mechanism linking lifestyle factors and colon carcinogenesis.

Introduction

Colorectal cancer risk is associated with diets high in fat and energy and low in complex carbohydrates and a sedentary lifestyle (1–6). Insulin resistance and non-insulin-dependent diabetes risk are associated with diet and exercise in the same way (7, 8). McKeown-Eyssen (9) and Giovannucci (10) have recently suggested that the two associations are indeed related. They proposed that diet and exercise factors lead to insulin resistance and hyperinsulinemia and that the increased level of insulin acts as a colon tumor promoter, perhaps through its action as a growth factor. Human and experimental animal studies have shown that diets high in fat, energy, and rapidly absorbed carbohydrates together with low levels of physical activity lead to insulin resistance, impaired glucose tolerance, and a compensatory increase in the levels of insulin (7, 8, 11–17). McKeown-Eyssen *et al.* (18) have recently presented preliminary evidence from a case-control study showing that colon polyp and cancer patients have higher levels of insulin as well as triglycerides and abdominal obesity compared to colonoscopy controls. However, to our knowledge, there has been no direct study showing that insulin is a promoter of colon cancer.

We report here on the promoting effect of insulin. Colon cancer in male Fischer 344 rats was initiated with AOM.[3] Insulin was administered exogenously in a manner that we considered would simulate the high levels of insulin after boluses of rapidly absorbed carbohydrates in animals resistant to insulin. The number of colon tumors in these animals was compared to the number in animals injected without exogenous insulin.

Materials and Methods

Animals and Diet. Sixty male Fischer 344 rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing approximately 180 g were housed in pairs in plastic cages with corncob bedding. The temperature and humidity were controlled at approximately 22°C and 50%, respectively. The room was maintained on 12-h dark/light cycles, with the dark cycle extending from noon until midnight. Purina Rodent Chow 5001 (Ralston Purina International, Strathroy, Canada) was provided *ad libitum* during the first nine days and pelleted AIN-93M (no. 110900; Dyets, Bethlehem, PA) was provided *ad libitum* for the remaining period of the study. Tap water from an automated system was provided *ad libitum*. Care of the animals conformed to the guidelines of the Canadian Council on Animal Care, and the protocol was approved by the University of Toronto Animal Care Committee.

Experimental Design. After 2 weeks of acclimatization, the rats were initiated with AOM (Sigma, St. Louis, MO) at a dose of 15 mg/kg body weight between 10 and 11 a.m., once a week for 2 weeks. Two days after the last AOM injection, they were randomized into two groups. Five days later, one group was given normal saline (0.2 ml); the other was given insulin (Iletin II NPH insulin isophane pork; Eli Lilly, Scarborough, Canada) diluted with saline (0.2 ml) and injected s.c. in the scapular region. NPH insulin is a medium-acting insulin that peaks approximately 4–5 h postinjection and returns to basal values within approximately 8 h postinjection (19). During the first 5 days of injection, insulin dosage was gradually increased (5 units/kg during the first 2 injection days, 10 units/kg during the next 2 days, and 15 units/kg for the remainder of the experiment). Injections were given 5 times/week (Monday through Friday) between 10 and 11 a.m. Insulin dosage was recalculated after weekly weight measurements. All the rats were sacrificed at 132 days after the first AOM injection, by which time 1 insulin-injected animal had died, and 15 others had developed bloody feces.

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[3] The abbreviation used is: AOM, azoxymethane.

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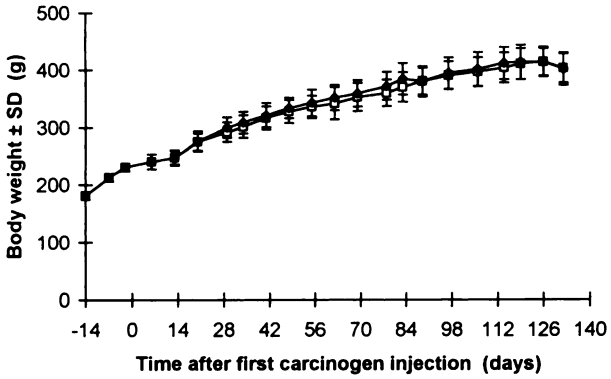

Fig. 1. Weekly body weights ± SD (g) of F344 rats during acclimatization (before day 0), AOM treatment (days 0 and 7), and subsequent injections of saline (□) or insulin (◆; days 14–132).

Blood Insulin and Glucose. Blood samples were collected by cardiac puncture under ketamine/acepromazine anesthesia (Vetrepharm, London, Canada; Ayerst Laboratories, Montreal, Canada) from 10 of the animals in both groups at 5 h after insulin or saline injections, 132 days after the first AOM injection. These samples were centrifuged after the addition of trasylol-EDTA (Miles Canada, Inc., Etobicoke, Canada) at 1800 rpm for 20 min. Insulin was measured in coded refrigerated aliquots by double-antibody RIA, and glucose was measured by the glucose-oxidase method (Vita-tech Canada, Inc., Markham, Canada).

Colon Tumor Assessment. Colons were removed, flushed with Krebs-Ringer bicarbonate buffer, cut lengthwise, spread flat on filter paper, and fixed in 10% buffered formalin for 9 days. The coded and randomized colons were then stained with 0.2% methylene blue according to the method of Bird (20) and examined for maximum tumor diameter and distribution throughout the colon, using light microscopy at ×40 magnification. Tumors with a maximum diameter of 2 mm or larger were examined histologically.

Statistics. A sample size of 30 rats/group was chosen to give a statistical power of 80% with a two-tailed P value of 0.05, assuming a 3-fold effect (tumor rate of 20% in the saline control and 60% in the insulin group). Following recommendations of Peto *et al.* (21), only colons obtained at the time of sacrifice were included in the analysis. Proportions of tumors were compared by the Fisher's exact test and χ^2 test. The number of tumors/animal, the maximum tumor diameter, and the distribution of tumors throughout the colon were compared using the two-tailed Student's t test as modified for unequal variance.

Results

Body Weights. There was no significant difference in the weekly mean body weights between the saline and insulin groups throughout the study period (Fig. 1).

Blood Insulin and Glucose. On the day of sacrifice at 5 h postinjection, serum insulin was significantly higher in the insulin group than in the saline group [488 ± 75 *versus* 47 ± 3 microunits/ml (mean ± SEM), respectively; $P < 0.001$]. The serum glucose was significantly lower in the insulin group than in the saline group [4.6 ± 0.4 *versus* 10.8 ± 0.7 mmol/liter (mean ± SEM), respectively; $P < 0.001$].

Colon Tumor Assessment. One rat in the insulin group died 120 days after the first AOM injection with bloody feces and a colonic tumor and was therefore excluded from the analysis. With light microscopy, 80 of the 81 tumors seen among all animals were confirmed as adenomatous polyps (tubular adenomas) with varying degrees of dysplasia (mild to marked). As shown in Table 1, the fraction of rats with confirmed colon tumors and the average number of tumors/rat were significantly greater in the group receiving insulin than in those receiving saline [for tumors ≥ 2 mm, 79 *versus* 50% ($P < 0.05$) and 2.00 *versus* 0.73 ($P < 0.001$), respectively]. There was no significant difference in the average tumor size between the two groups. The tumors were mostly in the distal colon; however, the distribution throughout the colon was not significantly different between the insulin and saline groups.

Discussion

We found that insulin given 5 days a week at a dose of 15 units/kg leads to increased levels of serum insulin, reduced levels of glucose, no effect on body weight, and a significant increase in the number of animals with colon tumors and the number of colon tumors/animal. The results show that insulin is a colon tumor promoter at these levels in this rat model. Our results thus support the role of high insulin levels in the development of colon polyps and cancer. Exogenous insulin has been used before in long-term animal studies with injection schedules similar to those used in this study. Rats, for instance, given insulin over a year have been found to develop atherosclerosis with increased endothelial and smooth muscle cell proliferation (19). This growth-promoting effect of insulin has also been observed in *in vitro* studies, in which added insulin has been found to lead to increased proliferation of a wide range of cells (22, 23). Diets elevated in fat, energy, and rapidly absorbed carbohydrates are known to increase endogenous insulin (7, 8, 11–17). The increased cellular proliferation observed with such diets (4, 24) may be a consequence of the exposure of the cells to increased endogenous insulin production. This assumption must be evaluated with systematic studies of the effect of experimental diets on insulin resistance and insulin levels on the one hand, and colonic proliferation and colon cancer promotion on the other.

McKeown-Eyssen and Giovannucci have suggested that insulin may have a tumor-promoting effect based on its known interaction with insulin and insulin-like growth factor-1 receptors (9, 10). Many human colon cancer tumors are known to express insulin and insulin-like growth factor-1 receptors (25–28). Insulin can act as a growth factor via these receptors, and repeated exposures to this stimulus might lead to the emergence of particularly responsive cells that would repopulate epithelium more rapidly than normal cells. However, insulin has a large number of effects throughout the body, and it is possible that the insulin-induced promotion is a consequence of changes of other hormones such as growth hormone or counter-regulatory hormones such as glucagon and corticosteroids. For instance, patients with acromegaly have a chronic hypersecretion of growth hormone and an increased risk of colon adenomas and cancer (29). Additional studies are needed to examine the sequence and role of insulin and its associated counter-regulatory hormones in the development of colon cancer.

The early yield of colonic adenomas in rats in this study is remarkable. Usually, AOM given once a week for 2 weeks at 15 mg/kg induces colon tumors including cancers after a period of approximately 225 days (2, 4). Here, an animal died with a tumor at 120 days, and many animals had tumors at 132 days. This study differed from the previous studies in one respect. Although AOM was given at the same time of the day, by the

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Table 1 Histologically proven colon tumors in rats injected with saline or insulin at 132 days after AOM initiation

Group	Total no. of rats	Rats with tumors (tumor incidence)	Total no. of tumors	Tumors/rat (Tumor multiplicity) (mean ± SEM)	Maximum tumor diameter (mean ± SEM) (mm)	Distribution throughout colon (mean ± SEM) (cm from rectal end)
Saline	30	15 (50%)	22	0.73 ± 0.16	4.0 ± 0.4	3.79 ± 0.58
Insulin	29[a]	23 (79%)[b]	58	2.00 ± 0.33[c]	4.5 ± 0.3	4.83 ± 0.41

[a] One rat in the insulin group died 120 days after the first AOM injection with a colonic tumor and was excluded from the analysis.
[b] Significantly different from corresponding saline group; $P < 0.05$ with Fisher's exact test and χ^2 test.
[c] Significantly different from corresponding saline group; $P < 0.001$ with two-tailed Student's t test as modified for unequal variance.

same route, and at the same dose as in earlier studies, the dark cycle was shifted earlier by 6 h. The advantage of this shift was that insulin levels peaked during the dark cycle when the rats ate more frequently and decreased their chance of hypoglycemic shock. However, this also meant that the animals reverted to the food-consuming state more quickly after the AOM injections and may have increased the effect of the AOM dose (30).

Finally, we should note a possible implication of our result on protocols for human intervention studies. The proposed model of colon carcinogenesis has two components: (*a*) first, as shown previously (7, 8, 11–17), diets high in fat and energy and low in complex carbohydrates in combination with low exercise levels increase the risk of insulin resistance and hyperinsulinemia; and (*b*) second, as shown here, elevated insulin levels lead to colon cancer promotion. If this model is valid for human colon carcinogenesis, diets and other lifestyle factors that reduce insulin would be expected to reduce colon cancer risk. Our results provide laboratory support for additional studies evaluating the efficacy of biomarkers of insulin resistance and hyperinsulinemia in the development of dietary, exercise, and possibly pharmaceutical strategies to reduce insulin-related cancer promotion and prevent colorectal cancer.

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