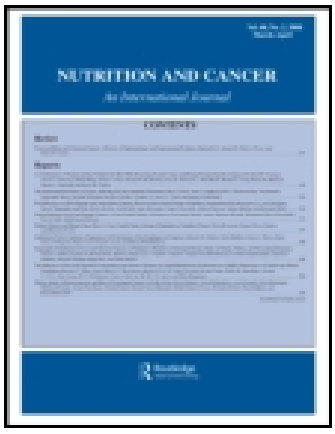

Nutrition and Cancer
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/hnuc20

Vaccenic Acid Feeding Increases Tissue Levels of Conjugated Linoleic Acid and Suppresses Development of Premalignant Lesions in Rat Mammary Gland

Sebastiano Banni , Elisabetta Angioni , Elisabetta Murru , Gianfranca Carta , Maria Paola Melis , Dale Bauman , Yan Dong & Clement Ip

Published online: 22 Jun 2011.

To cite this article: Sebastiano Banni , Elisabetta Angioni , Elisabetta Murru , Gianfranca Carta , Maria Paola Melis , Dale Bauman , Yan Dong & Clement Ip (2001) Vaccenic Acid Feeding Increases Tissue Levels of Conjugated Linoleic Acid and Suppresses Development of Premalignant Lesions in Rat Mammary Gland, Nutrition and Cancer, 41:1-2, 91-97, DOI: 10.1080/01635581.2001.9680617

To link to this article: http://dx.doi.org/10.1080/01635581.2001.9680617

NUTRITION AND CANCER, *41*(1&2), 91–97

Vaccenic Acid Feeding Increases Tissue Levels of Conjugated Linoleic Acid and Suppresses Development of Premalignant Lesions in Rat Mammary Gland

Sebastiano Banni, Elisabetta Angioni, Elisabetta Murru, Gianfranca Carta, Maria Paola Melis, Dale Bauman, Yan Dong, and Clement Ip

Abstract: *The objective of this report was to determine whether vaccenic acid (t11–18:1) is converted efficiently to conjugated linoleic acid (c9,t11–18:2, CLA) in rats via the Δ^9-desaturase reaction and, if so, whether vaccenic acid could substitute for CLA as an anticancer agent. In Study 1, rats were fed 1%, 2%, or 3% vaccenic acid in their diet, and tissue levels of CLA and CLA metabolites were determined in liver and mammary gland. In general, concentrations of CLA and CLA metabolites increased proportionately with an increase in vaccenic acid intake, at least up to the 2% dose level. Beyond this dose, there was clearly a plateauing effect. Thus vaccenic acid concentration increased from an undetectable level in the control to 78.5 nmol/mg lipid in the liver of rats fed a 2% vaccenic acid diet. This was accompanied by an increase in CLA from 2.3 to 33.6 nmol/mg lipid. These changes were also mirrored in the mammary gland, where increases in vaccenic acid (from 27.5 to 163.2 nmol/mg lipid) and CLA (from 17.8 to 108.9 nmol/mg lipid) were similarly observed. Vaccenic acid at 2% produced a CLA concentration in the mammary gland that was historically associated with a positive response in tumor inhibition based on our past experience. This provided the basis for selecting 2% vaccenic acid in Study 2, which was designed to evaluate its efficacy in blocking the development of premalignant lesions in the rat mammary gland. In this experiment, formation of histologically identifiable pathology due to intraductal proliferation of terminal end bud cells of mammary epithelium was used as the end point of analysis at 6 wk after carcinogen administration. Treatment with vaccenic acid reduced the total number of these premalignant lesions by ~50%. We hypothesize that the anticancer response to vaccenic acid is likely to be mediated by its endogenous conversion to CLA via Δ^9-desaturase.*

Introduction

The rumen microbial biohydrogenation of linoleic acid (*c*9,*c*12-octadecadienoic acid) to stearic acid (octadecanoic acid) involves the formation of conjugated linoleic acid (*c*9,*t*11-octadecadienoic acid, CLA) and vaccenic acid (*t*11-octadecenoic acid) as intermediates (1). In this report, we restrict the designation of CLA to only the *c*9,*t*11-CLA isomer. All other related isomers will be spelled out according to the positions of the conjugated diene as exemplified by *t*10,*c*12-CLA. Also, the term vaccenic acid refers specifically to the *trans* monoene fatty acid, and not the *cis* monoene isomer. When biohydrogenation in the rumen is incomplete, a portion of CLA and vaccenic acid may escape from the rumen and is taken up by tissues. Additional CLA can be synthesized from vaccenic acid in tissues via the Δ^9-desaturase reaction (2). These biochemical processes collectively contribute to the presence of vaccenic acid and CLA in cow's milk. Both of these fatty acids can be greatly enhanced in milk and milk-derived products if cows are fed a plant oil high in linoleic acid (3). Using the above procedure, Bauman and colleagues (4) produced a custom-made butter that is suitable for laboratory animal feeding studies. The predominant CLA in the butter is the *c*9,*t*11 isomer, and we previously reported that this CLA-enriched butter has potent anticancer activity in a rat mammary tumor model (5).

Chemical analysis of the CLA-enriched butter showed that vaccenic acid accounts for as much as 10% of total fatty acids (4,5). In view of our finding that rats fed the CLA-enriched butter accumulated considerably more CLA in their tissues than those fed the same level of a synthetic preparation of *c*9,*t*11-CLA (5), we speculated that vaccenic acid in butter may be an important precursor for the endogenous formation of CLA. This supposition has since been confirmed with the use of pure vaccenic acid in mice by the detailed biochemical studies of Santora et al. (6). Although there has been some concern about the health effects of *trans* fatty acids in the human diet, it should be noted that the epidemiological evidence is related to the wide spectrum of *trans* fatty acid isomers derived from the partial hydrogena-

S. Banni, E. Angioni, E. Murru, G. Carta, and M. P. Melis are affiliated with the Dipartimento di Biologia Sperimentale, Sezione di Patologia Sperimentale, Universita degli Studi di Cagliari, Cittadella Universitaria, 09042 Monserrato, Cagliari, Italy. D. Bauman is affiliated with the Department of Animal Science, Cornell University, Ithaca, NY 14853. Y. Dong and C. Ip are affiliated with the Department of Experimental Pathology, Roswell Park Cancer Institute, Buffalo, NY 14263.

tion of vegetable oils, and not to *t*11-octadecenoic acid (or vaccenic acid) present in ruminant food products (7).

We were interested in finding out whether vaccenic acid is also converted efficiently to CLA in rats (the species used in our cancer prevention research) and, if so, whether vaccenic acid could substitute for CLA as an anticancer agent. If a sufficient amount of CLA can be biosynthesized from vaccenic acid, it is reasonable to expect that the efficacies of vaccenic acid and CLA in cancer prevention would be quite similar to each other. The above rationalization forms the basis of our experimental approach. With this in mind, we set up a vaccenic acid dose-response experiment to determine the effects on tissue levels of CLA and CLA metabolites. The analysis was done in liver and mammary gland, because the liver is a major organ of fatty acid metabolism, and the mammary gland is the target site of our cancer research. On the basis of the tissue fatty acid data, we then selected a dose of vaccenic acid to examine its efficacy in blocking the development of premalignant lesions in the mammary gland. Because of the prohibitively high cost of pure vaccenic acid, it is not possible to use the conventional mammary cancer model similar to that described in our previous publications (8–11). Suffice it to note that we have evidence showing that the premalignant pathology data closely parallel the cancer data (12). Therefore, the premalignant lesion is a valid end point for evaluating the effectiveness of a cancer intervention agent. An advantage of this model is that it involves a much shorter treatment period and fewer animals. It is particularly desirable when the supply of reagent is limited.

Materials and Methods

Protocol of Vaccenic Acid Feeding

Female Sprague-Dawley rats were purchased from Charles River Breeding Laboratories (Raleigh, NC) at 45 days of age. They were fed a modified AIN-76 basal diet (13) for several days to acclimatize them to the powdered ration. The modification involved substituting the usual 5% corn oil with 5% butter fat. The latter was chosen to maintain a low background level of vaccenic acid and CLA in the control animals. The fatty acid composition of the butter fat was reported previously (4). Specifically, this particular batch of butter fat contained ~1.2% vaccenic acid and 0.5% CLA. A diet consisting of 5% butter fat by weight would provide 0.06% vaccenic acid and 0.025% CLA on a total ingredient basis.

To begin the vaccenic acid dose-response experiment, rats were divided into four groups (n = 4/group); the first continued to receive the basal diet, and the remaining three groups received an escalating dose of vaccenic acid at 1%, 2%, or 3%. Vaccenic acid of >97% purity was purchased from Nu-Chek Prep (Elysian, MN) and was mixed in the basal diet at the expense of an equivalent amount of carbohydrate. This dose range was chosen on the basis of the expectation that it would result in tissue CLA levels that are normally found in rats treated with CLA to achieve a cancer-preventive response. The four groups of rats were maintained for 3 wk. Livers and mammary fat pads were removed at necropsy, dropped in liquid nitrogen, and stored at −80°C until they were ready for analysis.

Lipid Analysis

Total lipid was extracted by the method of Folch et al. (14). Free fatty acids were obtained by a mild saponification procedure described by Banni et al. (15) and collected in *n*-hexane. After solvent evaporation, the residue was redissolved in CH_3CN-0.14% CH_3COOH (vol/vol) for injection into the high-performance liquid chromatography (HPLC) system. Separation of unsaturated fatty acids was carried out with a liquid chromatograph equipped with a diode array detector (model 1100, Hewlett-Packard, Palo Alto, CA). A C_{18} inertsil 5 ODS-2 column (Chrompack International, Middleburg, The Netherlands), 5-μm particle size and 150 × 4.6 mm, was used with a mobile phase of CH_3CN-H_2O-CH_3COOH (70:30:0.12, vol/vol/vol) at a flow rate of 1.5 ml/min (16). Nonconjugated diene unsaturated fatty acids were detected at 200 nm and conjugated diene unsaturated fatty acids at 234 nm. This method enables the determination of all unsaturated fatty acids, with or without a conjugated diene structure, in the same HPLC-ultraviolet detection system. Spectra (195–315 nm) of the eluate were obtained every 1.28 s and were electronically stored. Second-derivative ultraviolet spectra of the conjugated diene fatty acids were generated using the Phoenix 3D HP Chemstation software (Hewlett-Packard). These spectra were taken to confirm the identification of the HPLC peaks. Details of the methodology regarding the characterization of conjugated diene unsaturated fatty acids in reference and biological samples have been published (16). Statistical analysis was done by analysis of variance with post hoc comparisons, as described previously (11).

The distribution of CLA in several lipid fractions of the liver was also determined. Separation of major lipid classes was carried out according to the method described by Pietsch and Lorenz (17) with slight modifications. Briefly, samples containing ~5 mg of total lipid in 0.5 ml of chloroform were placed on an aminopropyl bond LRC column (Chrompack International) for solid-phase extraction. Each lipid class was eluted using different solvents: 5 ml of chloroform-isopropanol (2:1) for neutral lipid, 5.5 ml of acetic acid-diethyl ether (2:98) for free fatty acid, 20 ml of acetonitrile-*n*-propanol (2:1) for phosphatidylcholine, 7 ml of methanol for phosphatidylethanolamine, and 7.4 ml of isopropanol-3 N HCl in methanol (9:1) for phosphatidylinositol.

Protocol of Mammary Gland Premalignant Lesion Study

For the induction of premalignant intraductal proliferations (IDPs) in the mammary gland, rats were injected with methylnitrosourea at 50 mg/kg body wt ip when they were

50 days of age. Immediately after carcinogen administration, animals were divided into three dietary groups ($n = 6$ rats/group): control (fed 5% butter fat basal diet as described above), 2% vaccenic acid, or 1% *c*9,*t*11-CLA (Matreya, Pleasant Gap, PA). The 1% CLA group served as the positive control, since we previously reported the inhibition of IDP development by this treatment (12). Rats were fed these different diets for 6 wk. The abdominal-inguinal mammary gland chains on both sides were excised in one piece, fixed in methacarn, and processed in a Tissue-Tek Vacuum Infiltration Processor (Miles Scientific, Elkhart, IN). Each mammary gland whole mount was divided into six segments and embedded into paraffin blocks. Ribbons (5 μm thick) were cut from each block and placed on slides that had been treated with 3-aminopropyltriethoxysilane. Every 10th section was heat immobilized, deparaffinized in xylene, rehydrated in descending grades of ethanol (100% to 70%), and stained with hematoxylin and eosin. These hematoxylin-and-eosin slides were examined under the microscope for the appearance of IDP lesions using the criteria described by Russo and co-workers (18). Once a section showing the pathology of an IDP was found, the in-between slides were similarly stained to confirm the histology. The size of each IDP lesion could thus be estimated operationally by the number of serial sections showing the same pathology. The IDP data were analyzed by the χ^2 test using a Poisson regression model (19).

Results

Study 1: Vaccenic Acid Feeding and Tissue Lipid Analysis

Table 1 shows the dose response to vaccenic acid of the accumulation of vaccenic acid, CLA, and CLA metabolites in the liver. The metabolism of CLA (also designated CD 18:2, the CD prefix indicates the presence of a conjugated diene structure in the fatty acid) to CD 18:3, CD 20:3, and CD 20:4 by desaturase and elongase enzymes has been documented previously (20). No vaccenic acid (*t*11-18:1) was detectable in the liver of control rats. The feeding of 1% or 2% vaccenic acid, however, raised the concentration of this fatty acid to 34.9 or 78.5 nmol/mg total lipid, respectively. For some unexplained reason, a dose of 3% vaccenic acid depressed the level to 44.9 nmol/mg lipid. With respect to tissue concentration of CLA (CD 18:2), there was an 8- to 15-fold increase with the feeding of 1% or 2% vaccenic acid. The concentration of CLA seemed to plateau beyond this point. A similar pattern was also observed with the CLA metabolites including CD 18:3, CD 20:3, and CD 20:4. Thus it appears that the conversion of vaccenic acid to CLA (and subsequently to other downstream CLA metabolites) is maximized at 2% vaccenic acid in the diet.

The distribution of CLA in different lipid fractions of the liver from the above experiment was further investigated (Table 2). At each vaccenic acid dose level, neutral lipid had the highest concentration of CLA on a per milligram lipid basis. Phosphatidylcholine and phosphatidylethanolamine also contained significant amounts of CLA, while phosphatidylserine and phosphatidylinositol contained less. Thus it appears that CLA formed by the endogenous desaturation of vaccenic acid is incorporated primarily into neutral lipid, and secondarily, into various classes of phospholipids. This pattern is very similar to that observed when CLA is fed directly to the animals, as we recently reported (21).

Table 3 examines the effect of vaccenic acid feeding on other unsaturated fatty acids in the liver. These include palmitoleic (16:1), oleic (18:1), linoleic (18:2), γ-linolenic (18:3), dihomo-γ-linolenic (20:3), and arachidonic (20:4) acid. There were minimal perturbations of palmitoleic or oleic acid, even at the 3% vaccenic acid dose. In contrast, a ≥2% vaccenic acid reduced significantly the concentrations of linoleic acid and linoleic acid metabolites. These effects were especially marked at 3% vaccenic acid, resulting in a reduction of 36% for linoleic, 21% for γ-linolenic, 47% for dihomo-γ-linolenic, and 50% for arachidonic acid.

In general, the mammary tissue data paralleled closely the liver data. As shown in Table 4, the concentrations of vaccenic acid, CLA, and CLA metabolites increased proportionately with an increase in vaccenic acid intake, at least up to a dose of 2% in the diet. Beyond this dose, there was clearly a plateauing effect. In the mammary tissue, the total concentration of the three CLA metabolites (i.e., CD 18:3 + CD 20:3 + CD 20:4) represented only a small fraction of the CLA concentration (15–18% among the 4 treatment groups). This was different from the pattern in the liver,

Table 1. Dose Response of Vaccenic Acid Feeding on the Accumulation of Vaccenic Acid, CLA, and CLA Metabolites in Liver[a,b]

	Tissue Concentration, nmol/mg lipid				
Treatment	*t*11-18:1	CD 18:2	CD 18:3	CD 20:3	CD 20:4
Basal diet[c]	0*	2.3 ± 0.2*	0*	2.6 ± 0.3*	7.6 ± 1.3*
+1% Vaccenic acid	34.9 ± 2.3†	18.8 ± 3.0†	2.2 ± 0.3†	10.9 ± 1.0†	23.6 ± 2.2†
+2% Vaccenic acid	78.5 ± 3.6‡	33.6 ± 2.1‡	4.5 ± 0.5‡	14.9 ± 0.8‡	33.2 ± 1.2‡
+3% Vaccenic acid	44.9 ± 3.2†	26.8 ± 2.3‡	3.7 ± 0.4‡	16.9 ± 1.6‡	35.0 ± 4.2‡

a: Values are means ± SE ($n = 4$). CLA, conjugated linoleic acid; CD, conjugated diene.
b: Values with different symbols (*, †, ‡) are significantly different from each other ($P < 0.02$).
c: Basal diet (control) contained 0.06% vaccenic acid and 0.025% CLA.

Table 2. Distribution of CLA in Different Lipid Fractions in Liver of Rats Fed Different Levels of Vaccenic Acid[a,b]

Treatment	CLA Concentration, nmol/mg total lipid					
	NL	FFA	PC	PE	PS	PI
Basal diet[c]	0.8 ± 0.1*	0.1 ± 0.2*	0.4 ± 0.1*	0.3 ± 0.1*	0.2 ± 0.1*	0
+1% Vaccenic acid	5.8 ± 0.6†	0.5 ± 0.1†	2.6 ± 0.4†	2.1 ± 0.4†	0.8 ± 0.1†	0.1 ± 0.01
+2% Vaccenic acid	19.4 ± 2.0‡	1.2 ± 0.2‡	4.5 ± 0.2‡	3.5 ± 0.2‡	1.5 ± 0.1‡	0.2 ± 0.03
+3% Vaccenic acid	15.0 ± 1.1‡	0.8 ± 0.1†,‡	4.1 ± 0.5‡	3.1 ± 0.5‡	1.5 ± 0.1‡	0.1 ± 0.02

a: Values are means ± SE ($n = 4$). NL, neutral lipid; FFA, fatty free acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol.
b: Values with different symbols (*, †, ‡) are significantly different from each other ($P < 0.02$).
c: Basal diet (control) contained 0.06% vaccenic acid and 0.025% CLA.

Table 3. Effect of Vaccenic Acid Feeding on Accumulation of Palmitoleic, Oleic, Linoleic, γ-Linolenic, Dihomo-γ-Linolenic, and Arachidonic Acids in Liver[a,b]

Treatment	Tissue Accumulation, nmol/mg lipid					
	16:1	18:1	18:2	18:3	20:3	20:4
Basal diet[c]	143 ± 17	675 ± 33	216 ± 11*	5.7 ± 0.2*	100 ± 6*	328 ± 18*
+1% Vaccenic acid	168 ± 17	714 ± 37	217 ± 7*	6.0 ± 0.4*	91 ± 8*	307 ± 17*
+2% Vaccenic acid	177 ± 20	635 ± 52	175 ± 3†	5.5 ± 0.2*	60 ± 5†	198 ± 10†
+3% Vaccenic acid	142 ± 4	597 ± 21	139 ± 9‡	4.5 ± 0.4†	53 ± 3†	166 ± 10‡

a: Values are means ± SE ($n = 4$).
b: Values with different symbols (*, †, ‡) are significantly different from each other ($P < 0.02$).
c: Basal diet (control) contained 0.06% vaccenic acid and 0.025% CLA.

Table 4. Dose Response of Vaccenic Acid Feeding on Accumulation of Vaccenic Acid, CLA, and CLA Metabolites in Mammary Fat Pad[a,b]

Treatment	Tissue Concentration, nmol/mg lipid				
	*t*11-18:1	CD 18:2	CD 18:3	CD 20:3	CD 20:4
Basal diet[c]	27.5 ± 1.6*	17.8 ± 0.4*	0.51 ± 0.04*	1.9 ± 0.2*	0.12 ± 0.02*
+1% Vaccenic acid	75.0 ± 7.2†	58.9 ± 2.7†	1.6 ± 0.2†	7.5 ± 1.0†	0.55 ± 0.1†
+2% Vaccenic acid	163.2 ± 4.0‡	108.9 ± 7.1‡	2.9 ± 0.3‡	15.8 ± 0.9‡	1.2 ± 0.1‡
+3% Vaccenic acid	140.7 ± 11.2‡	112.1 ± 5.2‡	2.8 ± 0.3‡	15.9 ± 1.3‡	1.3 ± 0.1‡

a: Values are means ± SE ($n = 4$).
b: Values with different symbols (*, †, ‡) are significantly different from each other ($P < 0.02$).
c: Basal diet (control) contained 0.06% vaccenic acid and 0.025% CLA.

where the total concentration of the three CLA metabolites was always higher than the concentration of CLA itself. Because the mammary tissue consists predominantly of neutral lipid-rich adipocytes, we did not fractionate the mammary tissue lipid into different phospholipid classes for CLA distribution analysis.

The feeding of up to 2% vaccenic acid had little effect on the concentrations of the other unsaturated fatty acids in the mammary tissue. These results are summarized in Table 5. At 3% vaccenic acid, the concentrations of palmitoleic and oleic acids were decreased by 25% and 32%, respectively ($P < 0.02$), perhaps because of a decrease in Δ^9-desaturase activity. These changes, however, were not extended to linoleic acid or its downstream metabolites 18:3 and 20:4. Thus our data seemed to indicate that, compared with the liver, the mammary fat pad responded differently to vaccenic acid perturbation of the incorporation of other unsaturated fatty acids.

Study 2: Vaccenic Acid Feeding and Inhibition of Mammary Gland Premalignant Lesions

On the basis of our previous experience in mammary cancer with synthetic or natural CLA in the rat model, we routinely found that it was necessary to raise mammary tissue CLA to 70–100 nmol/mg lipid to obtain a positive response in tumor inhibition (5,10,11). The results in Table 4 show that 2% vaccenic acid in the diet was able to produce a CLA concentration of ~100 nmol/mg lipid. Furthermore, this dose of vaccenic acid did not affect the unsaturated fatty acid composition of the mammary tissue (Table 5). These findings provided the basis for selecting 2% vaccenic acid for the mammary gland IDP inhibition study.

We recently published a series of color histological micrographs showing the pathology of IDP progression (22). At a few weeks after methylnitrosourea administration, cells

Table 5. Effect of Vaccenic Acid Feeding on Accumulation of Palmitoleic, Oleic, Linoleic, Linolenic, Eicosatrienoic, and Arachidonic Acids in Mammary Fat Pad[a,b]

	Tissue Accumulation, nmol/mg lipid					
Treatment	16:1	18:1	18:2	18:3	20:3n–9	20:4
Basal diet[c]	272 ± 12*	1,225 ± 20*	78 ± 6	1.1 ± 0.2	1.8 ± 0.2	5.1 ± 0.5
1% Vaccenic acid	262 ± 8*	1,115 ± 23*	90 ± 8	1.3 ± 0.2	1.6 ± 0.3	5.3 ± 0.5
2% Vaccenic acid	309 ± 14*	1,186 ± 21*	72 ± 9	1.1 ± 0.2	1.5 ± 0.1	4.9 ± 0.3
3% Vaccenic acid	205 ± 11†	834 ± 18†	86 ± 10	1.1 ± 0.1	1.5 ± 0.3	4.6 ± 0.5

a: Values are means ± SE (n = 4).
b: Values with different symbols (*, †) are significantly different from each other ($P < 0.02$).
c: Basal diet (control) contained 0.06% vaccenic acid and 0.025% CLA.

Table 6. Reduction in Number of Premalignant IDP Lesions by Vaccenic Acid or CLA in Mammary Gland of Rats Given MNU[a]

	Size Distribution of IDP						Total No. of IDPs[b]
Treatment	≤10 sections	11–20 sections	21–30 sections	31–40 sections	41–50 sections	>51 sections	
Control	4	7	12	15	11	8	57*
+2% vaccenic acid	2	6	8	6	5	3	30†
+1% *c*9,*t*11-CLA	2	4	7	8	4	2	27†

a: Values represent lesions from a total of 6 rats/group. IDP, intraductal proliferation; MNU, methylnitrosourea.
b: Values with different symbols (*, †) are significantly different from each other ($P < 0.02$).

at the tip and neck of the terminal end bud begin to proliferate and invade the cavity of the ductal structure. The development of the IDP lesion continues until the lumen of the duct is partially or completely occupied. As noted in **Materials and Methods**, the size of each IDP lesion was estimated by the number of serial sections exhibiting this typical pathology. Table 6 describes the IDP data in rats given vaccenic acid or CLA (positive control). All the lesions were categorized into six classes with each containing ≤10, 11–20, 21–30, 31–40, 41–50, or >51 serial sections. No significant differences were found by treatment in a given class, probably because of the small sample number in each category when the data were segregated; this reduced statistical power to detect significant differences due to treatment. The total number of lesions across all size classes was summed, and the data were analyzed by Poisson regression. There were 57 IDP lesions in a total of 6 rats in the control group. Treatment with vaccenic acid or CLA reduced the number of IDPs to 30 and 27, respectively ($P < 0.05$). This design was not meant to compare the dose-response efficacy between vaccenic acid and CLA. The novelty here is in the finding that an anticancer effect can be achieved by the feeding of vaccenic acid per se.

Discussion

As far as we are aware, this is the first study to show that the feeding of vaccenic acid is able to elicit a biological response in vivo. The outcome is manifested by a decrease of mammary gland premalignant lesions in carcinogen-treated rats. We are favoring the hypothesis that the effect of vaccenic acid is mediated primarily through its conversion to CLA, which has been demonstrated to be a potent anticancer agent in the mammary gland model (8–11). However, at this point, we cannot completely rule out the possibility that vaccenic acid may have an independent effect by itself. Awad and co-workers (23) showed that vaccenic acid was able to inhibit modestly the growth of HT-29 human colon cancer cells compared with an equimolar concentration of stearic acid. Several papers have reported that Δ^9-desaturase, the enzyme responsible for converting vaccenic acid to *c*9,*t*11-CLA, is sensitive to inhibition by cyclopropene fatty acids (2,24,25). We plan to design additional experiments with the objective of determining whether the anticancer effect of vaccenic acid can be negated by simultaneous treatment with a cyclopropene fatty acid at a dose level that will block the formation of CLA endogenously.

More than 20 years ago, Mahfouz et al. (26) and Pollard et al. (27) described the desaturation of *trans* monenes to *cis,trans* 18:2 derivatives by rat liver microsomal Δ^9-desaturase. Recently, a detailed quantitative recovery study was carried out by Santora et al. (6) in mice to determine the extent to which dietary vaccenic acid might contribute to the body's supply of CLA. On the basis of carcass analysis, they estimated that ~12% of vaccenic acid consumed during a 2-wk feeding period was retained as CLA. As a proportion of vaccenic acid stored in tissues available for bioconversion, ~50% of it was desaturated. The study of Santora et al. clearly indicates that vaccenic acid is an excellent precursor for the endogenous synthesis of CLA in rodents.

In the mouse study of Santora et al. (6), CLA produced by vaccenic acid desaturation was found only in neutral lipid. Their observation was in contrast to our results, which show that in the rat liver the increase in CLA due to vaccenic acid feeding was incorporated in all major phospholipids, although the largest fraction was found in neutral lipid (Table 2). A number of reasons may explain the discrepancy. First, CLA metabolic disposition may be different in mice and rats. Second, Santora et al. measured CLA in the whole carcass, which consists of a more abundant amount of neutral lipid than phospholipid. It is possible that the low level of phospholipid CLA was masked by the much higher level of neutral lipid CLA when the whole carcass was analyzed. Third, Santora et al. used a corn oil diet, which was rich in linoleic acid, whereas we used a butter fat diet that was low in linoleic acid (5). A high linoleic acid intake may drive CLA (supplied exogenously or synthesized endogenously) into neutral lipid because of competition of incorporation into phospholipids by linoleic acid (21).

The presence of a similar metabolic pathway for the conversion of vaccenic acid to CLA has been suggested in humans (28,29). With the use of various deuterated *trans*- or *cis*-18:1 fatty acids, Adlof and co-workers (30) studied their conversion to deuterium-labeled CLA in a single male subject. They found that *t*11-18:1 and *c*11-18:1 were converted to *c*9,*t*11-18:2 and *c*9,*c*11-18:2, respectively, presumably via the Δ^9-desaturase reaction. Obviously, what happens in the normal population cannot be generalized from the information provided by one subject. Nonetheless, the study of Adlof et al. has implications with respect to estimating the availability of CLA in the human diet. Vaccenic acid is a major *trans* monoene in milk fat (4). For the sake of argument, if the desaturation of vaccenic acid in humans is quantitatively similar to that in mice (6), the amount of tissue CLA could be significantly higher than that resulting from the dietary consumption of CLA alone.

The beneficial effect of CLA in cancer prevention in humans is unclear. Our research showing that CLA or vaccenic acid is able to suppress the formation of early premalignant lesions suggests that an assessment of the habitual, rather than the current, intake of foods rich in these fatty acids is critical in epidemiological studies. This is because cancer requires many years to develop, and therefore a past history of CLA exposure is essential. Another lesson is that, in delineating the relationship between CLA and cancer risk in humans, the determination of tissue CLA, rather than dietary CLA, as a marker of exposure is likely to be more sensitive because of potential variations in the metabolic conversion of vaccenic acid to CLA among individuals.

Acknowledgments and Notes

This work was supported by National Cancer Institute Grant CA-61763 and Roswell Park Cancer Institute Core Grant CA-16056, a grant from National Dairy Council (Rosemont, IL), and a grant from Ministero dell'Università e della Ricerca Scientifica e Tecnologia, Italy. Address correspondence to C. Ip, Dept. of Experimental Pathology, Roswell Park Cancer Institute, Elm & Carlton St., Buffalo, NY 14263. Phone: (716) 845-8875. FAX: (716) 845-8100. E-mail: Clement.Ip@roswellpark.org.

Submitted 16 April 2001; accepted in final form 7 August 2001.

References

1. Griinari JM and Bauman DE: Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. In *Advances in Conjugated Linoleic Acid Research*, Yurawecz MP, Mossoba MM, Kramer JKG, Pariza MW, and Nelson G (eds). Champaign, IL: Am Oil Chem Soc, 1999, pp 180–200.
2. Griinari JM, Corl B, Lacy SH, Chouinard PY, Nurmela KVV, et al.: Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. *J Nutr* **130,** 2285–2291, 2000.
3. Kelly ML, Berry JR, Dwyer DA, Griinari JM, Chouinard PY, et al.: Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. *J Nutr* **128,** 881–885, 1998.
4. Bauman DE, Barbano DM, Dwyer DA, and Griinari JM: Production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models. *J Dairy Sci* **83,** 2422–2425, 2000.
5. Ip C, Banni S, Angioni E, Carta G, McGinley J, et al.: Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. *J Nutr* **129,** 2135–2142, 1999.
6. Santora JE, Palmquist DL, and Roehrig KL: *Trans*-vaccenic acid is desaturated to conjugated linoleic acid in mice. *J Nutr* **130,** 208–215, 2000.
7. Willett W, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, et al.: Intake of *trans* fatty acids and risk of coronary heart disease among women. *Lancet* **342,** 581–585, 1993.
8. Ip C, Scimeca JA, and Thompson H: Effect of timing and duration of dietary conjugated linoleic acid on mammary cancer prevention. *Nutr Cancer* **24,** 241–247, 1995.
9. Ip C, Briggs SP, Haegele AD, Thompson HJ, Storkson J, et al.: The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet. *Carcinogenesis* **17,** 1045–1050, 1996.
10. Thompson H, Zhu Z, Banni S, Darcy K, Loftus T, et al.: Morphological and biochemical status of the mammary gland as influenced by conjugated linoleic acid: implication for a reduction in mammary cancer risk. *Cancer Res* **57,** 5067–5072, 1997.
11. Banni S, Angioni E, Casu V, Melis MP, Carta G, et al.: Decrease in linoleic acid metabolites as a potential mechanism in cancer risk reduction by conjugated linoleic acid. *Carcinogenesis* **20,** 1019–1024, 1999.
12. Ip C, Ip MM, Loftus T, Shoemaker S, and Shea-Eaton W: Induction of apoptosis by conjugated linoleic acid in cultured mammary tumor cells and premalignant lesions of the rat mammary gland. *Cancer Epidemiol Biomarkers Prev* **9,** 689–696, 2000.
13. Report of the American Institute of Nutrition Ad Hoc Committee on the Standards for Nutritional Studies. *J Nutr* **107,** 1340–1348, 1977.
14. Folch J, Lees M, and Sloane-Stanley GH: A simple method for the isolation and purification of total lipid from animal tissues. *J Biol Chem* **226,** 497–509, 1957.
15. Banni S, Day BW, Evans RW, Corongiu FP, and Lombardi B: Liquid chromatographic-mass spectromeric analysis of conjugated diene fatty acids in a partially hydrogenated fat. *J Am Oil Chem Soc* **71,** 1321–1325, 1994.
16. Banni S, Carta G, Contini MS, Angioni E, Deiana M, et al.: Characterization of conjugated diene fatty acids in milk, dairy products, and lamb tissues. *J Nutr Biochem* **7,** 150–155, 1996.
17. Pietsch A and Lorenz RL: Rapid separation of the major phospholipid classes on a single aminopropyl cartridge. *Lipids* **28,** 945–947, 1993.
18. Russo J, Tay LK, and Russo IH: Differentiation of the mammary gland and susceptibility to carcinogenesis. *Breast Cancer Res Treat* **2,** 5–73, 1982.

19. McCullagh P and Nelder JA: *Generalized Linear Models*. London: Chapman and Hall, 1989.
20. Banni S and Martin JC: Conjugated linoleic acid and metabolites. In *Trans Fatty Acids in Human Nutrition*, Sebedio JL and Christie WW (eds). Aberdeen, UK: Oily Press, 1998, pp 261–302.
21. Banni S, Carta G, Angioni E, Murru E, Scanu P, et al.: Distribution of conjugated linoleic acid and metabolites in different lipid fractions in the rat liver. *J Lipid Res* **42,** 1056–1061, 2001.
22. Ip C, Thompson HJ, and Ganther HE: Selenium modulation of cell proliferation and cell cycle biomarkers in normal and premalignant cells of the rat mammary gland. *Cancer Epidemiol Biomarkers Prev* **9,** 49–54, 2000.
23. Awad AB, Hermann T, Fink CS, and Horvath PJ: 18:1 n7 fatty acids inhibit growth and decrease inositol phosphatase release in HT-29 cells compared to n9 fatty acids. *Cancer Lett* **91,** 55–61, 1995.
24. Jeffcoat R and Pollard MR: Studies on the inhibition of the desaturases by cyclopropenoid fatty acids. *Lipids* **12,** 480–485, 1977.
25. Andianaivo-Rafehivola AA, Blond JP, Cao J, Gaydou EE, and Bezard J: Influence of cyclopropene fatty acids (Baobab seed oil) feeding on the in vitro Δ^9 desaturation of stearic acid in rat liver microsomes. *J Nutr Biochem* **4,** 92–96, 1993.
26. Mahfouz MM, Valicenti AJ, and Holman RT: Desaturation of isomeric *trans*-octadecenoic acids by rat liver microsomes. *Biochim Biophys Acta* **618,** 1–12, 1980.
27. Pollard MR, Gunstone FD, James AT, and Morris LJ: Desaturation of positional and geometric isomers of monoenoic fatty acids by microsomal preparations from rat liver. *Lipids* **15,** 306–314, 1980.
28. Fogerty AC, Ford GL, and Svoronos D: Octadeca-9,11-dienoic acid in foodstuffs and in the lipids of human blood and breast milk. *Nutr Rep Int* **38,** 937–944, 1988.
29. Salminen MM, Mutanen M, Jauhiainen M, and Aro A: Dietary *trans* fatty acids increase conjugated linoleic acid levels in human serum. *Nutr Biochem* **9,** 93–98, 1998.
30. Adlof RO, Duval S, and Emken EA: Biosynthesis of conjugated linoleic acid in humans. *Lipids* **35,** 131–135, 2000.