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Combinatorial Chemoprevention: Efficacy of Lovostatin and Exisulind on the Formation and Progression of Aberrant Crypt Foci

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Abstract. *Background: There are several advantages to combinatorial chemoprevention strategies over monotherapeutic approaches. Both the HMG-CoA reductase inhibitor (HRI) lovastatin (LOV) and the selective apoptotic antineoplastic drug (SAAND) exisulind (EXS) have shown remarkable chemopreventive effects in previous studies, in cell lines and limited studies in rodents. Here, experiments were designed to assess the potential use of these two compounds in combinatorial chemoprevention therapy, using two bio-assays in which inhibition of the carcinogen-induced preneoplastic lesions, aberrant crypt foci (ACF), was used to quantitate efficacy. Materials and Methods: ACF were induced by the carcinogen azoxymethane (AOM) in F344 rats by two sequential weekly i.p. injections at a dose of 15 mg/kg. F344 rats were fed seven experimental diets containing LOV @ 50 parts per million (ppm), EXS @ 100, 250 and 1000 ppm and combination diets containing EXS at 100, 250 and 1000 ppm, each combined with LOV @ 50 ppm. Quantification of ACF number and type (singlet, doublet, triplet and four or more) was performed on whole mounts of rat colons stained with 1.0% methylene blue. Results: During the initiation protocol, administration of LOV @ 50 ppm alone and the combination of LOV @ 50 ppm with EXS @ 1000 ppm significantly decreased the mean number of ACF when compared to the positive control by 49% and 47%, respectively; however EXS @ 250 ppm displayed tumor promoting effects by significantly increasing the mean number of ACF by 64%. The post-initiation protocol administration of EXS @ 100, 250 and 1000 ppm and the combinations of LOV @ 50 ppm with EXS @ 100 and 250 ppm significantly increased the mean number of ACF when compared to the positive control by 44%, 48%, 55%, 49% and 40%, respectively. Conclusion: LOV shows greater promise than EXS in fulfilling the role as a supplemental chemopreventive agent in combinatorial chemopreventive strategies for cancers such as colon cancer. EXS did not augment this activity, failing to enhance chemopreventive therapy in this animal model.*

Colorectal cancer can occur in any part of the colon or rectum and is the third most common malignancy in the United States (1). An estimated 147,000 new cases of colorectal cancer arise each year, with approximately 57,000 people dying of colorectal cancer annually (2). Our understanding of the intricate processes of colon carcinogenesis broadens each year, allowing earlier intervention and possible prevention of the disease. Chemoprevention is defined as the "use of specific natural or synthetic chemical agents to reverse, suppress, or prevent the carcinogenic progression to invasive cancer" (3). Therefore, administration of chemopreventive agents can prevent carcinogens from reaching their target, reduce the number of cells the carcinogen can reach, or interrupt critical molecular pathways in carcinogenesis, often corrupted by tumor cells (4).

Over the past two decades, data from clinical studies, animal models and epidemiological studies have shown that NSAIDs have significant cancer chemopreventive properties (5). For example, the NSAID, sulindac, has been the focus of many colorectal chemoprevention studies. Sulindac is of the indene acetic acid class and is a pro-drug (sulindac sulfoxide), which is bio-activated into two metabolites (sulfide and sulfone) primarily in the kidney and liver, but also in the colon by bacterial microflora (6). Once administered, sulindac is rapidly absorbed and is reversibly

Abbreviations: ACF, aberrant crypt foci; COX, cyclooxygenase; cGMP, cyclic guanosine 3',5'-monophosphate phosphodiesterase; cGMP PDE, cyclic guanosine 3',5'-monophosphate phosphodiesterase; EXS, exisulind; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; HRI, HMG-CoA reductase inhibitors; JNK1, c-Jun NH_2-terminal kinase 1; LDL, low-density lipoprotein; LOV, lovastatin; MEKK-1, mitogen-activated protein kinase kinase kinase; PKG, cGMP-dependent protein kinase; ppm, parts per million; SEK1, stress activated protein/ERK kinase 1.

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reduced to the sulfide metabolite, which displays anti-inflammatory properties by inhibiting both isoforms of the Cox enzymes, Cox-1 (constitutive form) and Cox-2 (inducible form), thereby inhibiting prostaglandin synthesis. Sulindac also undergoes irreversible oxidation to the sulfone metabolite which, in contrast to the sulfide form, is devoid of any anti-inflammatory and Cox inhibitory properties (8). For example, in a study by Reddy *et al.* (7), rats treated with the carcinogen azoxymethane (AOM) and administered sulindac sulfone (EXS) at 1000 and 2000 ppm had significantly fewer colonic adenomas and carcinomas compared to positive control rats. The PGE_2 levels in rats treated with EXS ranged from 78%-118% of the control levels. Additionally, colons of rats administered with EXS did not display any significant inhibition of Cox, lipoxygenase, or phospholipase A2. These data suggested that the chemopreventive properties of EXS may not rely on the reduction of prostaglandin levels in the target tissue (8). Sulindac sulfide has shown promising potential as a chemopreventive agent for colorectal cancer, but at such high costs of toxicity, resulting in severe gastrointestinal irritation and ulceration, that questions remain concerning the future of sulindac sulfide in clinical settings (9). In contrast, EXS, known to be devoid of any anti-inflammatory or Cox inhibitory properties, has been shown to prevent tumor formation and inhibit aberrant crypt foci (ACF) formation in rodent models of colon carcinogenesis by triggering apoptosis independent of p53 expression and K-ras mutational status (10-13,15). EXS induces apoptosis by inhibiting the enzyme cGMP PDE, resulting in a persistent elevation of intracellular levels of cGMP, an induction PKG, a decrease in β-catenin accumulation *via* PKG-mediated β-catenin phosphorylation, and a rapid and sustained activation of JNK1, SEK1 and MEKK-1 (13,14).

Although EXS has arguably proven itself to exhibit protective effects against colon cancer in both *in vitro* and *in vivo* studies, its chemopreventive effects in human FAP patients have not been as promising. Two phase I trials studying the effect of EXS on polyp number and size in familial adenomatous polyposis (FAP) patients suggested that, although the number of polyps stabilized during the course of the study, at no dose did EXS definitively induce a reduction in overall polyp numbers (9, 16). At best, EXS provided consistent evidence of regression of small polyps in these studies. Furthermore, EXS had no effect on cellular proliferation in adenomatous tissue during treatment and an increase in the apoptotic index was significant only after 6 months of therapy (16). Yet other aspects of EXS, such as high tolerability, give it potential as a chemopreventive agent and make it an ideal candidate for combinatorial chemopreventive studies. By combining EXS with another potential or established chemopreventive agent, the complementary arrangement might possibly result in a more efficacious therapeutic modality for the chemoprevention of colorectal cancer. One candidate agent for such administration in combination with EXS is the cholesterol reducing statin drug, lovastatin (LOV).

LOV is a HMG-CoA reductase inhibitor (HRI), which reversibly inhibits the hepatic enzyme HMG-CoA reductase (16). Inhibition of HMG-CoA reductase induces relatively large reductions in both normal and elevated LDL cholesterol levels by preventing formation of melavonate (MVA) from HMG-CoA. MVA metabolism yields a series of isoprenoids such as farnesylpyrophosphate and geranylgeranyl-pyrophosphate that elicit pleiotropic effects ranging from cholesterol synthesis to cellular proliferation. Interestingly, inhibition of MVA metabolism and ultimately isoprenoid formation by administration of HRIs has been shown to reduce cellular proliferation and induce apoptosis (16). For example, LOV has been shown to induce apoptosis in prostate cancer cells, lymphocytes, leukemia cells and hepatocytes in culture (17). However, the significant toxicity of LOV at high doses has also precluded its use as a monotherapy for cancers. Therefore, studies have focused on determining the potential *synergistic* role of LOV in augmenting the effects of standard chemopreventive and chemotherapeutic agents. One such combinatorial chemopreventive study showed that LOV augmented sulindac-induced apoptosis in three colon cancer cell lines (HCT-116, SW480 and LoVo) in a COX-independent manner (18). Apoptosis induction was confirmed by the decreased expression of bcl-2 and increased expression of bax (17). Moreover, an *in vivo* study using the same combination of LOV and sulindac demonstrated a 28% and 30% reduction (compared to control) of total number of ACF and number of ACF containing 4 or more crypts, respectively (18).

The current study was designed to evaluate whether the administration of LOV at a dose of 50 ppm combined with EXS at three doses (100, 250 and 1000 ppm) to rats treated with AOM would augment the effect of EXS in reducing ACF numbers, when compared to positive controls, in a synergistic and non-toxic manner. Another important aim of this study was to utilize the established biochemical knowledge of these two potential chemopreventive drugs in order to formulate a logical strategy for inhibiting chemically-induced colon cancer in a multi-mechanistic fashion.

Materials and Methods

Animals, diets, test agents and carcinogens. Male F344 rats were purchased at approximately 6 weeks of age from Harlan Sprague-Dawley (Wilmington, MA, USA). The rats were quarantined for 7 days and then placed in standard cages in a 12-hour light/dark cycle. Ambient temperature was maintained at 21°C with relative humidity at 50%. The animals had constant access to drinking water and during the quarantine period all animals had constant access to rodent chow. After the 7-day acclimatization period, the

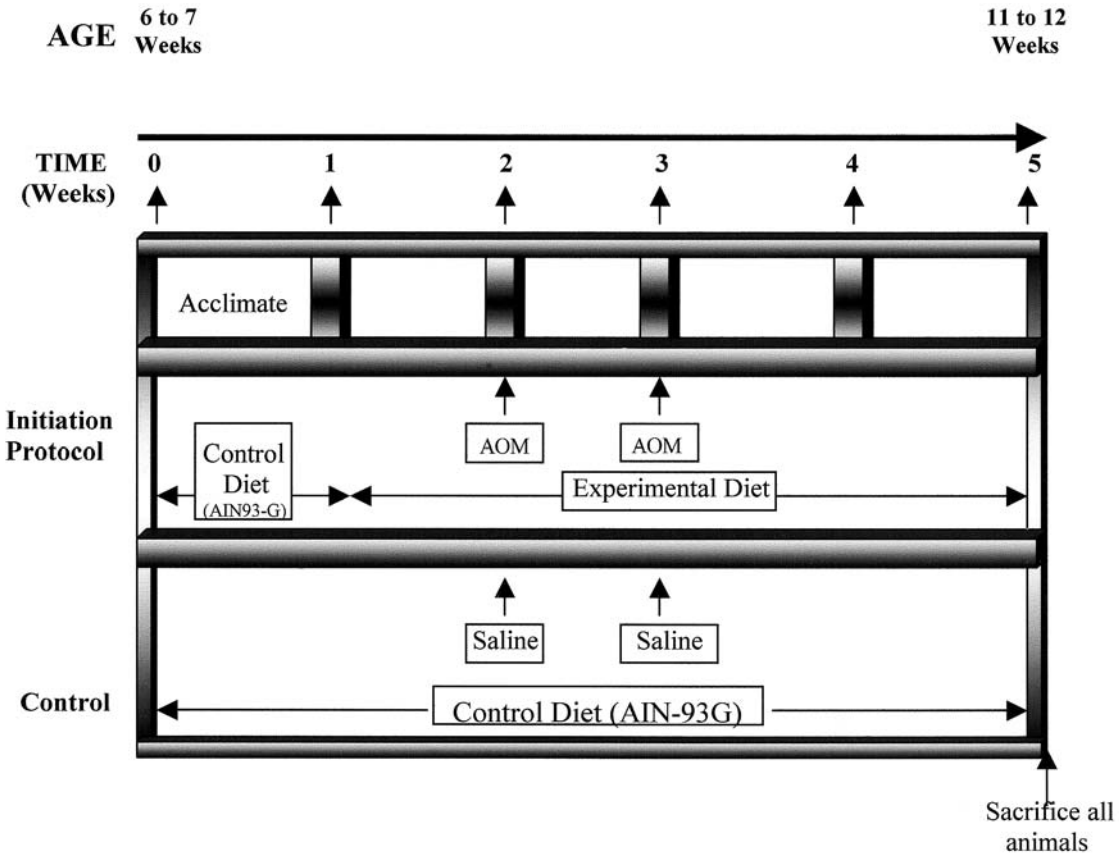

Figure 1. *Protocol A or initiation protocol. A 5-week study that tests the compounds' efficacy to interrupt the initiation of carcinogenesis.*

animals were placed on standardized AIN-93G diet without t-Butylhydroquinone (TBHQ) (Dyets, Inc., Bethlehem, PA, USA). All studies were approved by the Animal Care and Use Committee of the University of South Carolina, USA.

The animals were randomized and placed into 9 groups containing 3 or 10 rats, with either 2 or 3 rats per cage. The 9 groups were:
(i) negative control (no LOV and/or EXS, no azoxymethane (AOM) injections)
(ii) positive control (no LOV and/or EXS, with AOM injections)
(iii) LOV dosage in diet (LOV @ 50 parts per million (ppm), with AOM injections)
(iv-vi) EXS dosage in diet (EXS @ 100 ppm, 250 ppm and 1000 ppm, with AOM injections)
(vii-ix) Combined dosage of EXS and LOV in diet (EXS @ 100 ppm, 250 ppm, and 1000 ppm each combined with LOV @ 50 ppm, with AOM injections)

The positive and negative controls were fed standard AIN-93G diet throughout the duration of the experiments. The treatment groups had test agents mixed in with the standard, powdered AIN 93-G diet. LOV and EXS were provided by Cell Pathways Inc. (Horsham, PA, USA). These doses of LOV and EXS are not known to cause any signs of toxicity or cause significant loss of body weight in previous 5- and 8-week AOM experimental models, and were chosen based on communication with Cell Pathways Inc. All animals were weighed weekly to monitor any significant decrease in body weight.

Protocol A. Eighty-three rats (3 rats in group ii, 10 rats in the groups: i and iii-ix) were used in the initiation protocol (protocol A design; Figure 1). In protocol A, the experimental diets were given to the appropriate groups from the beginning of week 1 to the end of week 5. Stock experimental diets were prepared weekly and stored in a freezer at -20˚C. Experimental diets were dispensed through a food jar at approximately 20-22g of diet per rat per day. In protocol A, each rat in groups i and iii-ix were given a total of 2 doses of AOM (15 mg/kg of body weight; Sigma Chemical Company, St. Louis, MO, USA) *via* intraperitoneal injections, the first at the beginning of week 2 and the second at the beginning of week 3. Each rat in group ii (negative control) although not injected with AOM, did receive an equivalent volume of sterile saline *via* intraperitoneal injections. At the beginning of week 5, all rats were sacrificed by CO_2 asphyxiation immediately followed by manual decerebration. Each colon was then harvested for evaluation of ACF incidence.

Protocol B. Eighty-five (85) rats (15 in group i and 10 rats in each of the groups: iii-ix) were used in the post-initiation protocol (protocol B design; Figure 2).

In protocol B, the experimental diets were given to the appropriate groups (iii-ix) from the beginning of week 4 to the beginning of week 8. The procedures for preparation, administration and storage of experimental diets in protocol B were performed exactly as indicated for protocol A. AOM administration for all groups in protocol B occurred at the beginning of week 1 and

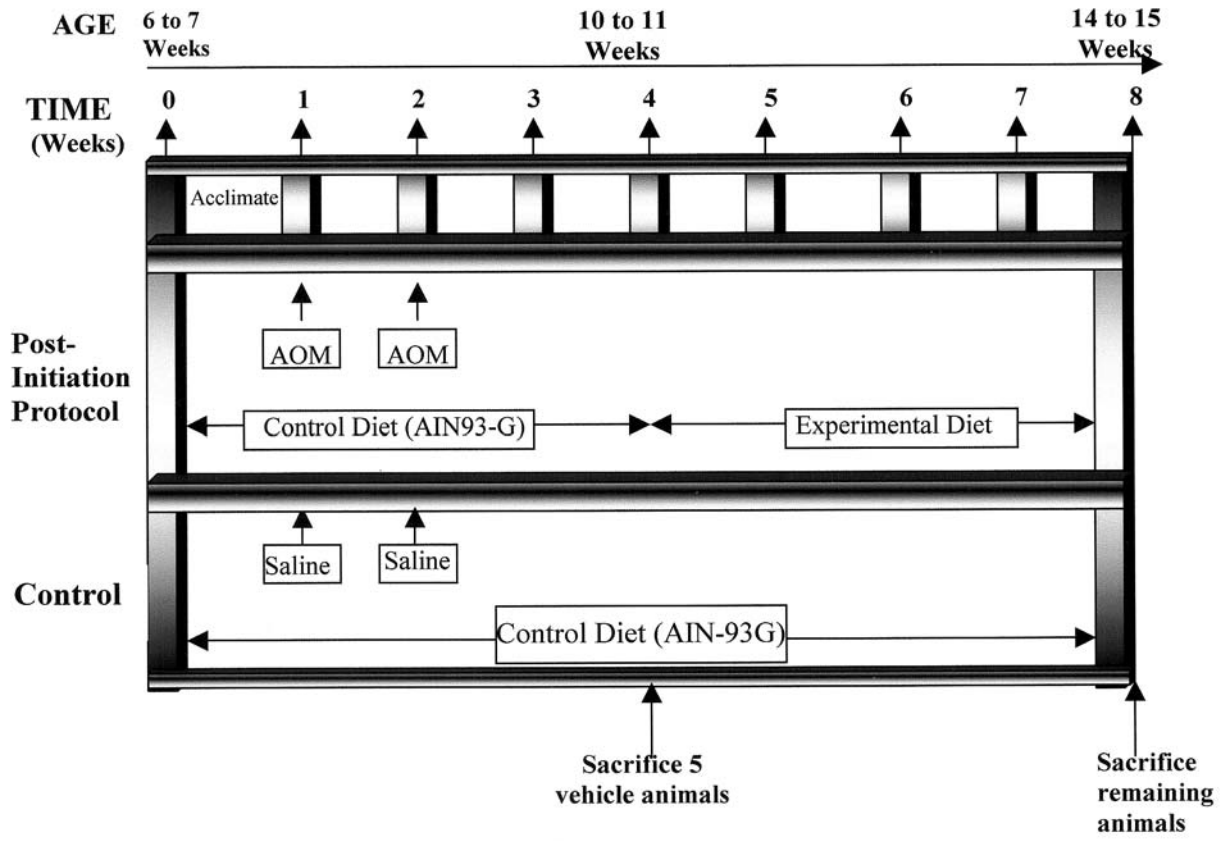

Figure 2. *Protocol B or post-initiation protocol. An 8-week study that tests the compounds' efficacy to interrupt the post-initiation phase of carcinogenesis.*

week 2 (number of dosages, doses and routes of administration are exactly as indicated in protocol A). At the beginning of week 4, 5 vehicle rats in group i were sacrificed and their colons were harvested for ACF evaluation. The remaining 80 rats were sacrificed for ACF evaluation at the beginning of week 8 in the same fashion as indicated for protocol A.

Aberrant crypt assay. The colons were removed and thoroughly flushed with cold phosphate-buffered saline (PBS). The colons were cut open along the longitudinal median exposing the mucosa side and stretched flat with forceps on an 8″ by 5″ piece of filter paper. The colons were then placed in 10% buffered formalin. The colons were stained with 1.0% methylene blue for approximately 45 to 60 seconds. After staining, the colons were washed vigorously in cold PBS to quantitate ACF. A single blinded scorer scored all colons. The colons were scored from distal to proximal end while recording the number of singlets, doublets, triplets and 4 or mores. ACF were distinguished as hyperplastic or dysplastic. All dyplastic ACF were scored, while hyperplastic ACF were not scored. The total number of ACF was also recorded and an average was compiled for each ACF type as well as a total average for each group.

Statistical analysis. All data were analyzed using Sigmastat 2.0 software and SPSS for Windows standard version 10.0.7 (SPSS, Chicago, IL, USA). Treatment and control groups were compared with each other using ANOVA. If the F-test of the ANOVA showed that at least one difference existed between the mean of the positive control and the means of the treatment groups, then the multiple comparison test, Dunnett's method, was used to explore which specific groups' means significantly differed from another at an alpha level of 0.05. The data were also tested for normality and equal variance. If the data did not exhibit a Gaussian distribution or failed to exhibit equal variance, a non-parametric one-way repeated measures ANOVA on ranks was performed. Statistical significance was given to data yielding a P value equal to or less than 0.05.

Results

The body weights of all animals in protocols A and B were measured once weekly throughout the experiments and were comparable to those of animals fed the control diet–AIN-93G (data not shown).

Efficacy of LOV @ 50 ppm and EXS @ 100, 250 and 1000 ppm alone or in combination on colonic ACF formation

Protocol A. In protocol A, the mean number of ACF counted in the positive control group was: 100 ± 12. Saline-treated rats had no ACF upon observation. Administration of LOV @ 50 ppm and various doses of EXS alone and in

Table I. *Total count of protocol A ACF and ACF types: singlet, doublet, triplet and 4 or more. Administration of agents resulted in a reduction of mean ACF per colon except for the administration of EXS @ 250 and 1000 ppm.*

Group	Singlet	Doublet	Triplet	4 >	Total	% of control.	% reduction
AOM Only	69 ± 7	22 ± 4	5 ± 2	1.5 ± 0.5	100 ± 12	100	0
LOV @ 50	37 ± 4*	12 ± 1*	1.3 ± 0.4	0.1 ± 0.1	51 ± 5*	51	49*
EXS @ 100	50 ± 4	17 ± 2	3 ± 0.5	1.2 ± 0.5	73 ± 5	73	28
EXS @ 250	119 ± 11#	37 ± 4*	6 ± 1	1.5 ± 0.7	164 ± 16#	164	64#
EXS @ 1000	79 ± 9	25 ± 4	4 ± 1	0.7 ± 0.4	109 ± 13	108	8
LOV @ 50 + EXS @100	64 ± 12	26 ± 4	7 ± 2	1 ± 0.4	97 ± 18	97	3
LOV @ 50 + EXS @ 250	53 ± 4	19± 2	4 ± 1	1 ± 0.4	79 ± 6	78	22
LOV @ 50 + EXS @ 1000	38 ± 5*	12 ± 2*	2 ± 0.3	0.6 ± 0.3	53 ± 7*	52	47*
No AOM, LOV @ 50 + EXS @ 1000	0 ± 0#	0 ± 0#	0 ± 0	0 ± 0	0 ± 0#	0	100#

* Significantly different from control group by Dunnett's method at: $p<0.05$.
\# Significantly different from control group by Dunnett's method at: $p<0.001$.

Table II. *Total count of protocol B ACF and ACF types: singlet, doublet, triplet and 4 or more. Administration of agents resulted in a elevation of mean ACF per colon except the administration of LOV alone and the combination of LOV with EXS @ 1000 ppm.*

Group	Singlet	Doublet	Triplet	4 >	Total	% of cont.	% reduction
Positive Control	21 ± 2	41 ± 2	22 ± 3	18 ± 1	102 ± 7	100	0
LOV @ 50	25 ± 3	35 ± 3	16 ± 2	17 ± 3	94 ± 9	92	8
EXS @ 100	42 ± 3#	57 ± 6*	22 ± 2	27 ± 3	147 ± 10*	144	44*
EXS @ 250	35 ± 2*	56 ± 2*	29 ± 2	31 ± 5*	151 ± 8#	147	48#
EXS @ 1000	42 ± 3#	59 ± 5*	29 ± 3	28 ± 3.	158 ± 9#	154	55#
LOV @ 50 + EXS @ 100	43± 4#	58 ± 3*	26 ± 2	26 ± 3	153 ± 8#	149	50#
LOV @ 50 + EXS @ 250	35 ± 2*	54± 3	28 ± 3	25 ± 3	142 ± 9*	140	40*
LOV @ 50 + EXS @1000	23 ± 2	30 ± 2	17 ± 1	17 ± 2	86 ± 5	84	16
No AOM, LOV @ 50 + EXS @ 1000	0 ± 0#	0 ± 0#	0 ± 0	0 ± 0	0 ± 0#	0	100#

* Significantly different from control group by Dunnett's method at: $p< 0.05$.
\# Significantly different from control group by Dunnett's method at: $p< 0.001$.

combination with LOV resulted in both reduction and elevation of ACF numbers (Table I).

An approximately 50% reduction of mean number of total ACF occurred with administration of LOV @ 50 ppm and the combination of LOV @ 50 + EXS @ 1000 ppm, while slightly lower reductions of 28%, 22% and 3% occurred with administration of EXS @ 100 ppm, LOV @ 50 + EXS @ 250 ppm and LOV @ 50 + EXS @ 100 ppm, respectively. The intermediate dosage level of EXS @ 250 ppm inexplicably exacerbated the AOM induction of total ACF by approximately 64%, while a modest increase of 8% was observed with the administration of EXS @ 1000 ppm. Subsequent statistical analysis such as ANOVA and multiple comparison procedures were utilized to note the statistically significant differences of ACF numbers of treatment groups from control groups (Table I).

The effects of these two agents on ACF multiplicity is also shown in Table I. While LOV alone did inhibit the yield of some multiple crypt foci, the effect was not uniformly enhanced by co-treatment with EXS.

Protocol B. In protocol B, the mean number of ACF counted in the positive control group was: 102 ± 7 (Table II). Administration of LOV @ 50 ppm and various doses of EXS alone and in combination with LOV resulted in both reduction and elevation of ACF numbers. The action of LOV @ 50 ppm alone on total ACF incidence in protocol B was similar to that in protocol A in decreasing total ACF incidence, but to a lesser degree of 8%. LOV @ 50 + EXS @ 1000 ppm was approximately twice as effective as LOV alone in decreasing total ACF numbers by 15%. The remaining groups all exacerbated the carcinogenic effects of AOM in a range of 40% to 55%.

Analysis of yields of total ACFs revealed that EXS @ 100, 250 and 1000 ppm and the combinations of LOV @ 50 + EXS @100 ppm and LOV @ 50 + EXS @ 250 ppm all

increased the mean number of total ACF to a statistically significant degree. For the remaining treatment groups, LOV @ 50 ppm and LOV @ 50 + EXS @ 1000 ppm, any reduction of the mean number of total ACF observed when compared to the positive control was not statistically significant.

Multiple comparison analysis of the mean number of ACF singlets yielded similar results to that of total ACF. A statistically significant increase was observed in the mean number of ACF singlets of each of the three independent EXS doses and the combinations of LOV @ 50 + EXS @ 100 ppm and LOV @ 50 + EXS @ 250 ppm.

Discussion

The hypothesis under study was that administration of LOV @ 50 ppm combined with EXS @ 100, 250 and 1000 ppm to rats treated with AOM would augment the chemopreventive activity of EXS, reducing ACF numbers when compared to positive controls, and do so in a synergistic and non-toxic manner. However, the data from both protocols do not provide validity to the hypothesis and, also, EXS unexpectedly and inexplicably enhanced ACF yields. Thus, the data suggest that LOV does not augment the chemopreventive effects of EXS in a synergistic fashion.

Inhibition of ACF due to LOV @ 50 ppm was expected and supported data from a similar study conducted by Agarwal *et al.* (18), which also found LOV @ 50 ppm to decrease ACF incidence. On the other hand, since no previous studies have observed the effects of LOV combined with EXS, the 47% significant reduction of mean total ACF exhibited by the combination of LOV @ 50 + EXS @ 1000 ppm was most probably due entirely to LOV.

In protocol A, the chemopreventive effects of LOV @ 50 ppm alone and of the combination of LOV @ 50 + EXS @ 1000 ppm decreased ACF incidence to approximately the same degree (2% difference). The data suggest that administration of LOV @ 50 ppm alone is as effective a potential chemopreventive agent as the combination of LOV @ 50 + EXS @ 1000 ppm. Moreover, in protocol A, none of the three independently administered doses of EXS (100, 250, 1000 ppm) resulted in a significant reduction in ACF incidence, which suggests that the role of EXS in inhibiting ACF formation may be minimal to none. Thus, it is possible that LOV does not synergistically augment the chemopreventive effects of EXS, but instead maintains its own independent ability to actively inhibit ACF formation.

Data from protocol A also suggested that one experimental treatment significantly increased the ACF incidence. EXS @ 250 ppm increased mean total ACF by 64% in protocol A, indicating that EXS at 25% of its MTD seems to promote colonic ACFs. *In vitro* studies have demonstrated that EXS induces apoptosis in colon cancer cell lines (7). However, EXS, albeit accepted as a pro-apoptotic agent, lacks significant chemopreventive activity in the current study, either alone or in combination.

No experimental diet in protocol B significantly reduced the mean number of ACF. In contrast, five experimental groups: EXS @ 100, 250, 1000 ppm and the combination of LOV @ 50 + EXS @ 100 ppm and LOV @ 50 + EXS @ 250 ppm exhibited tumor promoting activity by increasing ACF incidence to a significant degree.

Not all studies utilizing EXS as an anti-neoplastic agent have found EXS to be efficacious. Stoner *et al.* (9) found EXS to show no effect on cellular proliferation under basal *versus* mitogenically-stimulated conditions. Additionally, *in vivo* studies using the APC^{Min} mouse model, which shares the APC mutation associated with patients who have familial adenomatous polyposis (FAP), have shown EXS to have no significant effect on adenoma numbers (15). Finally, clinical trials using EXS therapy for a minimum of 6 months in order to observe the effect of EXS on polyp formation in FAP patients found that doses of 200, 600 and 800 mg/day treatment with EXS did not reduce polyp number. However, the same study also concluded that, after at least 6 months of EXS therapy, at best polyp numbers did stabilize and a significant increase in the apoptotic index, as well as evidence of regression of smaller polyps, was observed (16)

Potential variables that may have influenced the results may involve a matter of scorer's bias. However, the possibility that scorer's bias affected the results is minimal since all scoring was conducted with a blinding method. Moreover, protocol A and B's positive control numbers differed by 2%

A key strategy learned from the efforts of the past few decades of biomedical research is that frequent and reliably effective treatments rarely utilize a monotherapeutic approach and that combinatorial strategies can provide significant improvements over single agent therapies. The potential role of EXS as a combinatorial chemopreventive agent seems promising when reviewing the results from *in vitro* studies, yet does not exhibit its potential as effectively in *in vivo* and clinical studies. Contradictory results and technical problems from *in vivo* and clinical studies challenge the value of subsequent investigations focusing on the role of EXS as a combinatorial chemopreventive agent. Our results also cast doubt on the chemopreventive efficacy of EXS in experimental colon models.

In contrast, LOV has displayed promising results in many arenas of chemoprevention. Not only did LOV exhibit a chemopreventive efficacy in this study, but previous studies have confirmed its potential as a versatile chemopreventive and therapeutic agent (17-22). LOV can potentially increase

tumor cell kill and minimize adverse effects associated with chemotherapeutic and radiotherapeutic regimes with its ability to selectively target tumor cells or cells manifesting the codon 12 K-ras mutation, and restore or induce the cells' sensitivity to apoptosis while sparing normal cells (20). Therefore, the combination of LOV or other HRIs such as simvastatin or atorvastatin in conjunction with other cancer preventive agents should not be overlooked. Moreover, LOV and other statins are widely used in the elderly for prevention of coronary heart disease. Thus, the application of LOV may not be confined only to the prevention and treatment of heart disease, but may possibly extend to include supplementation to tumor therapy or colon cancer prevention in the main group of current elderly statin users who are also at high risk of developing neoplasias (21). In these patients LOV could serve a dual function of lowering blood cholesterol levels and augmenting the effectiveness of chemopreventive therapies as a combinatorial chemopreventive agent. An ideal agent would be efficacious and non-toxic when combined with drugs such as LOV. EXS, however, failed to produce enhanced chemopreventive effects when combined with LOV in the present study and other agents should be considered.

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