Development of Bowman-Birk Inhibitor for Chemoprevention of Oral Head and Neck Cancer

FRANK L. MEYSKENS, JR.

Department of Medicine and Chao Family Comprehensive Cancer Center, University of California, Irvine, Orange, California 92868, USA

ABSTRACT: Leukoplakia in the oral cavity has been used as a putative surrogate marker of head and neck cancer development. A class of chemoprevention compounds, called protease inhibitors, has been shown *in vitro* and in animal models to effectively suppress premalignant lesions. Bowman-Birk inhibitor (BBI) is a protease inhibitor derived from soybeans that has demonstrated chemoprevention activity in many *in vitro* and animal systems, including the hamster cheek pouch model. Pilot, Phase I and Phase IIa studies of Bowman-Birk Inhibitor in patients with oral leukoplakia have demonstrated no detectable side effects. In the Phase IIa trial, changes in the protease activity in oral mucosal cells after BBI Concentrate© (BBIC) treatment correlated with the changes in neu protein levels. Additionally, evidence for a dose-related treatment effect of BBIC on oral leukoplakia was demonstrated. These results indicate that BBIC should be investigated for chemopreventive activity in a randomized clinical trial.

KEYWORDS: Bowman-Birk inhibitor; oral leukoplakia; oral head and neck cancer

INTRODUCTION

There exists a large amount of epidemiologic and experimental data that suggests protease inhibitors have significant anticarcinogenic activity.[1] Several different types of protease inhibitors have been shown to inhibit the carcinogenic process, and on a molar basis those that inhibit chymotrypsin proteases have been the most effective. The soybean-derived chymotrypsin protease inhibitor known as the Bowman-Birk inhibitor (BBI) has been the most widely studied. The structure of BBI is shown in FIGURE 1. It is a 71-amino-acid protein, and its structure was delineated in 1973.[2] The presence of five cystine bridges and both chymotrypsin and trypsin inhibitory sites is a unique feature of this molecule. A great deal of biochemical and biological work has been done with this protein, and it was recognized early that removal of the trypsin-inhibitable activity in soybeans was important as toxicity was largely related to this property of extracted proteins. It was also recognized that improper preparation resulted in ineffectiveness of the chymotrypsin-inhibitable function as well.

Address for correspondence: Dr. Frank L. Meyskens, Jr., Department of Medicine and Chao Family Comprehensive Cancer Center, University of California, Irvine, 101 The City Drive South, Orange, CA 92868. Voice: 714 456-6310; fax: 714 456-2240.
FLMeyske@uci.edu

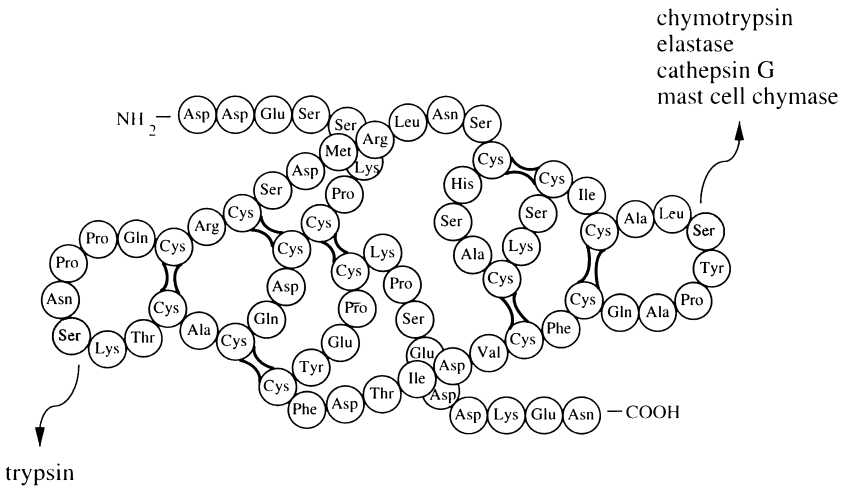

FIGURE 1. Structure of Bowman-Birk Inhibitor. The chymotrypsin inhibitory site appears on the right side (Leu–Ser) and the trypsin inhibitor site appears on the left side (Lys–Ser) from Odani and Ikenaka.[2] (Reproduced from Kennedy[1] with permission.)

Eventually an extract from soybeans (BBI Concentrate©) with the same ability as BBI to inhibit carcinogenesis was developed.[3] This was an important achievement since pure BBI is extremely expensive to obtain and its complex internal structure precludes a simple synthesis and, to date, the development of a biosynthetic engineering approach. BBIC is very stable, maintaining its chymotrypsin-inhibitable activity and its ability to inhibit transformation *in vitro* for over two years.[4] BBIC was approved by the FDA in 1992 for Investigational New Drug status and clinical trials were begun at that time. Our initial single-dose trial of the inhibitor demonstrated that the compound was well tolerated and without side effects, up to doses of 800 chymotrypsin inhibitory units (CIU), the highest dose tested.[5]

INTERMEDIATE MARKERS OF ORAL CARCINOGENESIS

The development of risk and surrogate intermediate markers for oral cancer has been a lengthy, difficult, and time-consuming process.[6,7] We have investigated the role of a number of potential intermediate markers in oral carcinogenesis and have

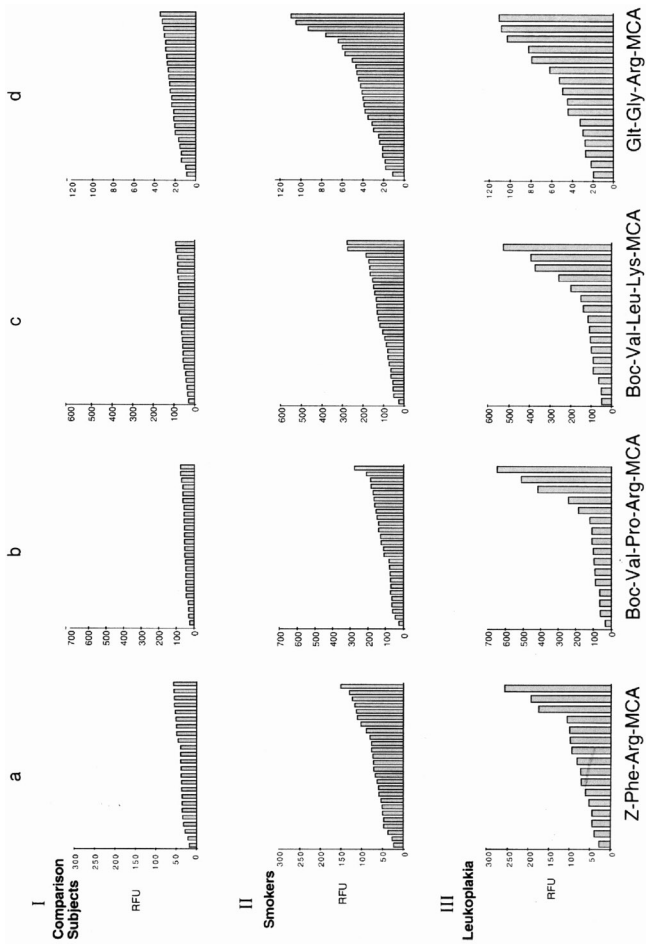

FIGURE 2. Levels of proteolytic activities in the oral buccal mucosa cells. I, 26 comparison subjects; II, 41 smokers; and III, 20 patients with leukoplakia. Data are expressed as the relative fluorescent units (RFU) per µg protein. RFU are determined for each of four substrates [Z-Phe-Arg-MCA (**a**), Boc-Val-Pro-Arg-MCA (**b**), Boc-Val-Leu-Lys-MCA (**c**) and Glt-Gly-Arg-MCA (**d**)] based on the absorbance of the MCA reporter group relative to that of 10^{-7}_{M} MCA standard. data for each subject group are plotted on the same scale and show the consistent low level of protease activity for each substrate among comparison subjects (20 males and 6 females) of various ages. Patients with leukoplakia and smokers have much more variable levels of proteolytic activities in their buccal mucosa cells, and the levels may be much higher than that of comparison subjects. (Reproduced from Manzon *et al.*[8] with permission.)

determined that proteolytic activities and neu oncogene expression are the most promising. We have reported that levels of proteolytic activities are increased 2- to 3-fold in oral mucosa cells of smokers and patients with oral leukoplakia or erythroplakia as compared to a non-smoking comparison group (see FIGURE 2).[8] Elevated levels were also found in patients with oral trauma and in diabetic patients as well as pregnant women. Since synthetic substrates are used to make these measurements reproducibility is high.

Elevated levels of neu oncogene protein on cancer cells have been demonstrated in many epithelial cancers.[9] This molecule is a 185,000 dalton protein (p185) and is also referred to as c-erb-B-2 and Her-2. Serum neu protein is derived from the extracellular domain of the neu protein, which is released by proteolytic cleavage from the cell surface. A detailed study was done to determine the relationship between protease activity and neu protein levels in patients with oral leukoplakia.[10] A number of correlations were demonstrated: pretherapy serum and cellular neu protein levels were related, but the protease activity in oral mucosal cells did not correlate with either parameter. However, after BBIC treatment, activities of the cellular protease activity did correlate with serum neu levels (see FIGURE 3). A comparison of the changes in these markers pre- and post-treatment showed that the change in both serum and cellular neu protein correlated with changes in the protease activity. Overall, these results suggested that BBIC inhibited cleavage of the neu protein on the cell surface. This observation raises the possibility that by inhibiting the cleavage of neu protein on the cell surface BBI may work by preventing premalignant cells from escaping immunological surveillance and elimination of neu protein antigen.

Thirty-two patients were studied in our one-month, Phase IIa clinical trial of BBIC.[11] No consistent individual or group clinical side effects were detected and no effect on serum micronutrients demonstrated. Clinical response was determined by measurement and comparison of pre- and post-treatment individual lesion areas (as well as the summed total in each patient) and analysis of blinded clinical judgments of scrambled photographs. Two patients achieved a complete clinical response and an additional eight achieved a partial clinical response. The clinical response data are summarized in TABLE 1. Overall, a 24.2% increase in total lesion area was observed following treatment and a linear fit of the dose-response relationship between dose of BBIC and decrease in total lesion area was evident. Additionally, no toxicity to the BBIC was detected, up to the maximal dose tested (1066 CIU). We also found that high pretreatment oral mucosal cell protease activity was associated with a greater decrease in protease activity after BBIC administration. Since both the level of protease activity and its response to BBIC affected response, we have modeled these parameters on clinical response and the optimized model is shown in FIGURE 4.

FUTURE STUDIES

The results from these series of clinical trials are highly encouraging. To date, BBIC has not produced detectable clinical or laboratory toxicity, even at the highest doses tested. We have shown evidence in a non-randomized trial for shrinkage of oral leukoplakia lesions and modulation of cellular protease and serum and cellular neu protein. We have now embarked on a longer placebo-controlled and randomized

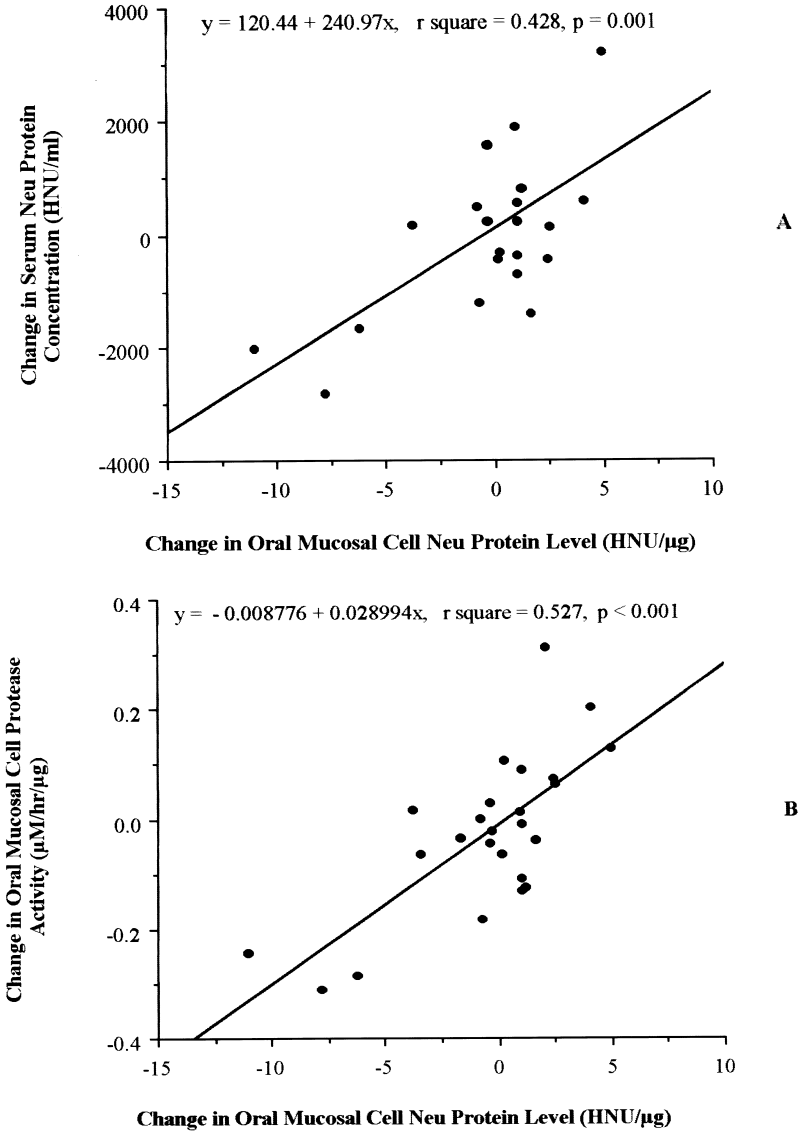

FIGURE 3. See opposite page.

Phase IIb trial in which clinical lesion size, cellular protease activity, and serum and cellular neu protein levels will be measured and changes correlated. The broad involvement of the neu protein in many cancers suggests that exploration of BBIC in the prevention and treatment of other cancers would be worthwhile as well.

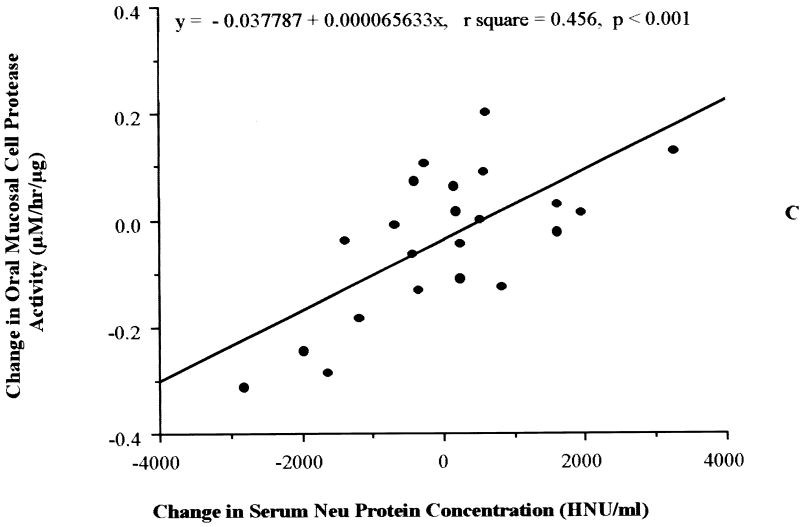

FIGURE 3. Correlation between the changes in levels of oral mucosal cell neu protein, serum neu protein concentration, and oral mucosal cell protease activity after BBIC treatment. The neu protein levels in oral mucosal cells and in serum and the Boc-Val-Pro-Arg-MCA hydrolysis activity in oral mucosal cells were determined in 25 patients with oral leukoplakia before and after the 1-month treatment with BBIC. The relationship between changes in the levels of neu protein and protease activity after BBIC treatment were analyzed by a linear regression analysis. The neu protein measurement and protease assay were carried out in duplicate. (Reproduced from Wan *et al.*[10] with permission.)

TABLE 1. Clinical response of BBIC with respect to dose administered

Dose	Prog[a]	NR[b]	PR[c]	CR[d]	*N*	Response
200	0	7	1	0	8	12.50
533	0	7	3	1	11	36.36
800	2	5	2	0	9	22.22
1066	0	1	2	1	4	75.00
Total	2	20	8	2	32	31.25

[a]Progression (Prog): Appearance of new lesions, or greater than 50% increase in total lesion area.
[b]No Response (NR): Less than 50% decrease in size to 50% increase in size.
[c]Partial Response (PR): at least 50% reduction in total area of all lesions.
[d]Complete Response (CR): Complete resolution of all lesions at completion of one month BBIC.
(Reproduced from Armstrong[11] with permission.)

FIGURE 4. Modeled BBIC dose-clinical response relationship following multiple regression analysis. Simultaneous least-squares multiple regression was performed. Clinical response was modeled by an intercept, baseline PA, and dose to correct for initial PA level. This histogram shows the estimated clinical response to BBIC at each dose, assuming patients have the average initial value of PA (estimated from the data). (Reproduced from Armstrong *et al.*[11] with permission.)

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