PILOT INTERVENTION STUDIES WITH CAROTENOIDs

H.F. Stich\textsuperscript{a}, M.P. Rosin\textsuperscript{a}, A.P. Hornby\textsuperscript{a}, B. Mathew\textsuperscript{b}, R. Sankaranarayanan\textsuperscript{b} and M. Krishnan Nair\textsuperscript{b}

\textsuperscript{a}Environmental Carcinogenesis Unit
British Columbia Cancer Research Centre
Vancouver, B.C. V5Z 1L3
Canada

\textsuperscript{b}Regional Cancer Centre
Trivandrum 695011
India

INTERMEDIATE ENDPOINTS FOR INTERVENTION TRIALS

Considerable attention is currently being given to beta-carotene as a promising chemopreventive agent (1,2). Several observations can be cited in support of this idea. Epidemiological evidence points to an inverse relationship between the intake of beta-carotene-containing green or yellow vegetables and the incidence of cancers at various sites (3-7), including oral cancer (8). Animal studies show beta-carotene to have a marked protective effect against a variety of carcinogens (9,10), and in vitro experiments have revealed an antimutagenic (11) and antitransformation (12) activity. Whether these protective effects of beta-carotene are due to its scavenging potential for radicals (13,14), to its capacity to interfere with the activation of carcinogens, or to its conversion into vitamin A which is the actual chemopreventive agent, is at present difficult to assess. Several largescale intervention trials have been initiated to prove or disprove the usefulness of beta-carotene in preventing the development of carcinomas. However, clinical trials using cancer as the endpoint are expensive, require a relatively large number of participants, and last for a long time. Tests that could provide more rapid results would be invaluable in assessing treatment protocols before long-term, manpower-intensive intervention trials are initiated. Two "intermediate endpoints" appear to be worth a more detailed validation. Firstly, preneoplastic lesions, including dysplasia (15), polyps (16), leukoplakia (17), and esophagitis (18), have been successfully applied to track the response towards chemopreventive agents. In our pilot trial on betel quid chewers, we examined the effect of beta-carotene and beta-carotene plus vitamin A on the remission of established oral leukoplakias and the development of new ones. Secondly, the usefulness of micronucleated cells as markers in chemoprevention trials has been explored (19,20). Micronucleated cells appear to be good indicators for carcinogen-induced injuries to chromosome complements (21). Increased frequencies of micronucleated cells were observed in tissues at elevated risk for the development of cancer, including the oral mucosa of...
betel quid chewers (22), snuff dippers (20), users of various tobacco-containing mixtures, including Khaini tobacco (23) and nass (24), and cigarette smokers (25-27). Since micronuclei reveal the "pathobiologically effective dose" which is more indicative than a mere "exposure dose", they should provide information on the protective action of chemopreventive agents (28).

SHORT-TERM INTERVENTION TRIAL

Fishermen (Kerala, India) with oral leukoplakias, as defined by the WHO Collaborating Centre for Oral Precancerous Lesions (29), participated in the pilot intervention study. They all chewed a simple type of betel quid, consisting of areca nut (Areca catechu L.), tobacco (Nicotiana tabacum L.), slaked lime from marine shells (calcium hydroxide) and betel leaf (Piper betle L.). The participants in the trial were randomly allocated to three groups, one receiving beta-carotene (180 mg/week, given twice weekly in 6 capsules), another beta-carotene (180 mg/week) plus vitamin A (100,000 IU/week), and the third placebo capsules, all administered orally. Butylated hydroxyanisole and butylated hydroxytoluene, which are present in some forms of beta-carotene, were absent from the preparations used in this study.

The frequency of micronucleated exfoliated cells (MEC) from the buccal mucosa was elevated in all tobacco chewers who participated in the trial: 4.1 ± 1.5 (n=61), compared to 0.4 ± 0.2 (n=67) in non-chewers. There appeared to be no significant differences in MEC frequencies between cell samples taken from normal-appearing areas of the oral mucosa and those collected from regions with leukoplakias: 4.1 ± 1.5 in normal mucosa, and 3.9 ± 1.2 in areas with leukoplakias among the 61 betel quid chewers examined. The reduction of micronucleated cells following a 3-month oral administration of beta-carotene (180 mg/week) is shown in Figure 1. Two of the chewers did not respond to the treatment.

![Fig. 1. Frequency of exfoliated cells with micronuclei from a normal-appearing area of the oral mucosa of betel quid chewers (Kerala, India) before and after the oral administration of placebo or beta-carotene (4 months, 180 mg/week).](image-url)