Abstract. Background: Cigarette smoke (CS) is the main inducer of oral cancer, increasing its prevalence by 4-7 times. Materials and Methods: We examined the suitability of cell models SCC-25 and SCC-15 for studying effects of CS on oral cancer cells and whether CS significantly affects the cell cycle using fluorescence-activated cell sorting assays. Results: There was significant change in the fraction of SCC-15 cells in pre-G$_1$ state following CS exposure. At 60 and 90 min, increase in the pre-G$_1$ cell fraction was 118% (p<0.05) and 135% (p<0.01) respectively. The G$_2$/M cell fraction was significantly lower following CS exposure. At 90 and 120 min following CS exposure, the G$_2$/M fraction levels had declined by 44% (p<0.05) and 34% (p<0.01) respectively. Results for SCC-25 cells were similar. At 90 and 120 min following CS exposure, the pre-G$_1$ fraction of the cells increased by 230% and 550%, respectively (p<0.01). At 120 min of CS exposure, the fraction of G$_2$/M cells was lower by 47% (p<0.05) compared to controls. Conclusion: CS profoundly affects the cell-cycle distribution in both SCC-15 and SCC-25 oral cancer cellular models. Such effects have been associated with DNA damage and carcinogenesis. Both models are useful for studying oral cancer pathogenesis.

Squamous cell oral carcinoma is the sixth most prevalent type of cancer in the world, with approximately 30,000 new cases diagnosed annually in the United States and 200 in Israel. The overall 5-year survival rate for patients throughout the world is 50%, among the lowest for major types by cancer, and has not changed in the last half century (1-7). Despite therapeutic and diagnostic progress related to this cancer, the disease is characterized by a high rate of morbidity and the mortality rate remains unchanged. Thus, a better understanding over the biological nature of this aggressive disease is mandatory. It has been well-established that cigarette smoke (CS) is the main inducer of oral cancer, increasing its prevalence by 4-7 times (1-3).

The purpose of the current study was to examine the suitability of two cell models for studying the effects of CS on oral cancer cells. We intended to examine whether CS significantly affects the cell cycle of oral cancer cells in two widely used, but different oral cancer cell lines: SCC-25 and SCC-15, by using fluorescence-activated cell sorting (FACS) assays.

Materials and Methods

Cancer cell lines. In this study we used SCC-25 and SCC-15 human squamous cells, which are epithelial carcinoma cells of the tongue. These two cell lines are commonly used in oral cancer research. The cells (the American Type Culture Collection, Rockville, MD, USA) were grown in 90% Dulbecco’s Modified Eagle Medium: Nutrient Mixture (DMEM) Ham’s F-12 media. Cultures also contained 10% heat-inactivated fasting blood sugar (FBS), 2.5 mM L-glutamine, penicillin-streptomycin solution (10,000 units/ml penicillin sodium salt and 10 mg/ml streptomycin sulfate; 1% v/v). Cells were grown at 37°C in 95% air and 5% CO$_2$.

Exposure to CS. In order to expose cells to CS, a cigarette was combined with a vacuum system to enable the influx of gas-phase cigarette smoke into a sealed apparatus containing a Petri dish with the cells in 5-10 ml medium (depending on dish size). The cells had been cultured previously for 3 days until they were confluent. A reproducible vacuum was created in the apparatus and, upon opening the vacuum, a cigarette was lit for approximately 5 s. In this way 80-100 ml of cigarette smoke in ‘puffs’ entered the apparatus with a pressure of 0.2 bar (~150 mmHg). After part of the cigarette had been consumed, the dishes were further incubated with the smoke for 15 min at room temperature and then another influx was performed. This model mimics exposure of smokers’ mouths to CS. Cells that were used for controls were subjected to the same procedure, but exposed to fresh air rather than CS. The vacuum level was identical.

Fluorescence-assisted cell sorting (FACS). FACS was used to monitor the cell-cycle profile. Cells were trypsinized, centrifuged (1,000 × g for 5 min at 4°C), and the pellets re-suspended in 4 ml
phosphate-buffered saline (PBS) then centrifuged again (1000 × g for 5 min at 4°C). The pellet was re-suspended in 475 μl of a solution containing 0.3% saponin, 50 μg/ml RNase, 5 mM EDTA (pH 8), all the components dissolved in PBS (pH 7.4). After 30-min incubation at room temperature in the dark, 50 μl was added (0.5 μg/ml). After a further 10-min incubation in the dark, cells were vortexed briefly before FACS analysis. For each assay, 15,000 cells were randomly collected.

**Statistical analysis.** For statistical analysis, experimental and control groups were replicated at n ≥5. Results are presented as the mean±SD. Determination of statistical significance was performed using Student’s t-test. When required, one-way analysis of variance (ANOVA) was carried-out and the appropriate tests performed. The criterion for statistical significance was a p-value of less than 0.05.
Results

Cell-cycle analysis for the SCC-15 cell line. As can be seen in Figure 1a, there was a significant change in the fraction of SCC-15 cells in the pre-G1 state following CS exposure. At 60 min and at 90 min, the increase in the pre-G1 cell fraction was 118% (p<0.05) and 135% (p<0.01), respectively. The difference noted at 90 min remained similar at 120 min (Figure 1a). In contrast, the differences regarding the G1 cell fraction between the control and the CS-exposed cells were not significant (Figure 1b). The G2/M cell fraction was significantly lower following CS exposure. At 90 min and at 120 min, the G2/M fraction was reduced following CS exposure by 44% (p<0.05) and 34% (p<0.01), respectively (Figure 1c).

Cell-cycle analysis for the SCC-25 cell line. In general, similar results were obtained for the SCC-25 cells. Thus, at 90 min and at 120 min following CS exposure, the pre-G1 cell fraction of the cells increased by 230% and 550%, respectively (p<0.01) (Figure 2a). In contrast, the fraction of cells at G1 was basically not affected by CS, although a slight tendency for reduction was noted following CS exposure (Figure 2b). This reduction was also noted for the G2/M fraction, where it was more substantial. At 120 min of CS exposure, the fraction of G2/M SCC-25 cells was lower by 47% (p<0.05) as compared with controls (Figure 2c).

Discussion

Oral cancer kills one person every hour. The 5-year survival rate has remained at 50% for the past five decades in spite of the vast clinical research and progress relating to therapy. Such therapy is also accompanied by a significant level of morbidity. Accordingly, basic research focused on the underlying mechanism of oral cancer is mandatory, especially surrounding the role of CS in its pathogenesis (4-7). The purpose of the current study was to examine CS-induced effects on the cell cycle of two cellular models for oral cancer, SCC-25 and SCC-15 cell lines.

We evaluated cell-cycle changes derived from CS exposure and found that in both cell models, there was a significant decrease in the G2/M fraction (p<0.05) of the exposed cells and a much smaller decrease in the G1 fraction (not all statistically significant). For example, in SCC-25 cells there was a significant (p<0.01) change in the pre-G1 cell fraction following more than 90 min exposure to CS. There was tendency for the G1 and G2/M fractions to decrease in cells exposed for up to 90 min. This appears to indicate that there was little decrease in cell-cycle arrest. However, following 120 min exposure, there was a significant (p<0.05) decrease in the G2/M fraction. This reflects a shortening of the cell cycle, indicating a higher proliferative rate than in cells not exposed to CS.

These results are in accordance with those of other studies (8, 9), where it was shown that agents such as free radicals and ionizing radiation can trigger a significant decrease in the G2/M cell fraction in various cell lines. Interestingly, other researchers reported an increase in the G2/M fraction induced by CS or free radicals: Narayan et al. reported that a single-dose treatment of a normal breast epithelial cell line with CS resulted in a transformed phenotype (10). The anchorage-dependent growth of these cells decreased due to increased arrest of cells in the S-G2/M phase, suggesting that CS is capable of transforming these cells in vitro, supporting the role of cigarette smoking in increasing the risk for breast cancer. Esakky et al. recently reported that CS induces accumulation of spermatocytes at the S-G2/M phase of the cell cycle (11), while Drukteinis et al. found that the polyaromatic hydrocarbon benzo[alpha]pyrene, a major toxicant in CS, significantly inhibited proliferation in a dose-dependent manner, characterized by G2/M cell arrest in the human trophoblastic JEG-3 cell line (12). This antiproliferative effect of benzo[alpha]pyrene involves activation of a p53-dependent pathway with cell-cycle arrest at G2/M, providing evidence of oxidative stress and activation of a DNA-damage response pathway in JEG-3 cells.

In summary, our results demonstrate that CS profoundly affects the cell cycle in both SCC-15 and SCC-25 oral cancer cellular models.

References


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