INTRODUCTION

Flavonoids, a group of naturally occurring compounds found abundantly in fruits and vegetables, has recently received much attention in cancer chemoprevention. There have been numerous epidemiologic studies, laboratory studies, and human clinical trials indicating that flavonoids have important chemopreventative properties. The proposed mechanisms of action include carcinogen inactivation, cell cycle arrest, induction of apoptosis, promotion of differentiation, and inhibition of angiogenesis. Our current study utilizes two flavonoids, apigenin and kaempferol, that have been shown to effectively inhibit agonist binding to the aryl hydrocarbon receptor. This ligand activated cytosolic receptor translocates to the nucleus and regulates expression of target genes involved in procarcinogen activation. Apigenin and kaempferol inhibit cell proliferation in a malignant oral cell line both in vitro and in vivo.

METHODS

In vitro model

The experiments were conducted with FaDu cells, a well known human squamous cell carcinoma cell line established from a tumor of the upper aerodigestive tract. Following incubation for 24 hours the cells were treated with increasing concentrations (0.1, 1, 10, 25, 50, and 100 micromolar) of each kaempferol and apigenin. Following 24 and 48 hours of treatment cell viability was determined using the WST-1 assay. The data was analyzed using one-way ANOVA and Tukey’s multiple comparison tests.

In vivo model

Athymic nude mice at approximately 6 wks of age were used for the experiments. Three groups of nude mice (10 to 12 mice/group) were treated by gavage for one week prior to inoculation. Group 1 was treated with 50μg kaempferol per day via gavage. Group 2 was treated in the same fashion with 50μg apigenin per day via gavage. Group 3 or the control group was treated with corn oil in the same fashion. After 1 week of gavage the left flank of each nude mouse was injected subcutaneously with 0.1 ml (1x105 cells) of FaDu cell suspension. The right flank of each mouse served as control and was injected with 0.1 ml of Matrigel mixed with cell free culture medium. The mice were maintained under pathogen free conditions. Once the tumor became palpable, the volume was regularly monitored, measuring the length, width, and height of tumors. Tumors were weighed at sacrifice at 28 days after inoculation.

RESULTS

In vitro results

- Cell viability decreases in the 24 and 48 hour treated FaDu cells with increasing concentrations of both kaempferol and apigenin (p < 0.001).
- The response is more pronounced in the 48 hour treated cells.
- All conditions were significantly different from controls except the cells treated with Apigenin at 0.1 and 1 μM (Tukey p < 0.001).

In vivo results

- Apigenin group’s tumor volume was significantly higher than controls (p<0.03, average volume of 3024mm³ vs. 1858mm³).
- The Kaempferol group’s tumor volume was higher than controls (2610mm³ vs. 1858mm³), but this did not reach statistical significance (p=0.09).
- The tumor weights at time of harvest for the control group, kaempferol group, and apigenin control group were 1.08 grams, 1.66 grams, and 1.74 grams respectively.
- We saw no evidence of overt kaempferol or apigenin toxicity. Each group of test mice showed similar weight gain throughout the experiment.

CONCLUSION

- Treatment of oral squamous carcinoma cell lines with apigenin and kaempferol in culture results in a dose dependent reduction in cell viability.
- Apigenin and Kaempferol increase tumor growth in athymic mice.
- Translational work examining these flavonoids as SCCA chemopreventative agents would be unwise until this phenomenon is better understood.

FINANCIAL DISCLOSURES

This project was funded in part by NIH, NCI grant R03 CA125781 and a University of Kentucky Department of Surgery Research Grant.

REFERENCES