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13. ABSTRACT (Maximum 200 Words)

The disease of cancer is usually attacked at time of diagnosis, and even chemoprevention is not usually considered until adulthood. Our hypothesis is that windows of development hold the key for chemoprevention of prostate cancer. We have previously demonstrated that genistein is bioavailable to the rat prostate and that life-time exposure to physiological concentrations of genistein suppressed the development of chemically-induced prostate cancer. The purpose of our research is to determine if there is a developmental window for this chemoprevention and the mechanism(s) of chemoprevention. We have demonstrated that neonatal and prepubertal exposure to genistein via the diet does not alter development of the prostate buds in 21 and 35 day old rats. More recently, we have demonstrated that prepubertal, as well as adult and lifetime, exposure to genistein in the diet down regulated androgen receptor, but not estrogen receptors alpha and beta, expression in the dorsolateral prostate of rats. Down regulated androgen receptor is consistent with genistein suppressing prostate cancer development. Short term exposure to genistein having a permanent effect on androgen receptor expression is hypothesized to occur via androgen receptor promoter DNA methylation. Tumorigenesis studies are completed and are in the process of being evaluated for histopathology.

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Introduction

The disease of cancer is usually attacked at time of diagnosis, and even chemoprevention is not usually considered until adulthood. Our hypothesis is that windows of development hold the key for chemoprevention of prostate cancer. We have previously demonstrated that life-time exposure to physiological concentrations of genistein suppressed the development of chemically-induced prostate cancer. We have shown that genistein is bioavailable to the prostate. We have also demonstrated that genistein did not readily cross the placenta, hence we don't believe that the gestational period plays a significant role in the protective effect. On the other hand, it is primarily during the first weeks of postnatal life that prostate differentiation takes place, a period that may be influenced by genistein. The purpose of our proposed research is to determine if there is a developmental window for this chemoprevention and the mechanism (s) of chemoprevention. The importance of this lies in the need to know, prior to initiation of human trials, if we need to expose infants and/or adults to get maximum chemoprevention. We propose to accomplish this in a dietary model at "physiological concentrations".

Body

Aim 1. To determine if a specific window of development (prepubertal only, adult only or life-time) is responsible for genistein chemoprevention of prostate cancer. This will be done on the following groups of rats, 30/group initially.
Group A) genistein via the diet from birth throughout life to confirm that postnatal lifetime exposure only protects against prostate cancer.
Group B) genistein in the diet from birth until 35 days of age only.
Group C) genistein in the diet starting at 90 days of age, 20 days after cancer initiation.
Group D) no genistein in the diet as positive controls.

This was initiated in the middle of the first year. The breeders were purchased, the rats bred, treated with the carcinogenesis protocol, and exposed to genistein in the diet as listed above. The last of the rats for this aim were recently necropsied this month. The tissues have been fixed and the blocks are being sectioned. The slides will be submitted for hemotoxylin and eosin staining and sent to Dr. Eltoum for histopathologic evaluation. The histopathology report will be available in the beginning of year 3.

Aim 2. To investigate prostate gland morphology in the dorsal and lateral lobes of the prostates of 21 and 35 day old rats exposed ± genistein in the diet, starting at birth. Months 6-12. No statistical significance was detected for prostate bud perimeter from genistein compared to control treated rats. This work was completed in year 1 and has already been reported.

Aim 3. To investigate the potential of genistein to regulate sex steroid receptor expression as mechanism of prostate cancer prevention. This was projected to be carried out during year 2.

Dietary genistein resulted in decreased androgen receptor, but not estrogen receptors alpha or beta, expression in dorsolateral prostates (Figures 1 and 2). The novel finding is that prepubertal short term (days 1-35 postnatal) genistein exposure was able to down regulate the androgen receptor as well as did the adult only (days 56-70) and life time (days 1-70) genistein treatments. Since short term early exposure to genistein is as effective as short term late and lifetime exposure to genistein, this suggests that early exposure to genistein can have a programming or imprinting effect on androgen receptor expression. One mechanism by which genistein could

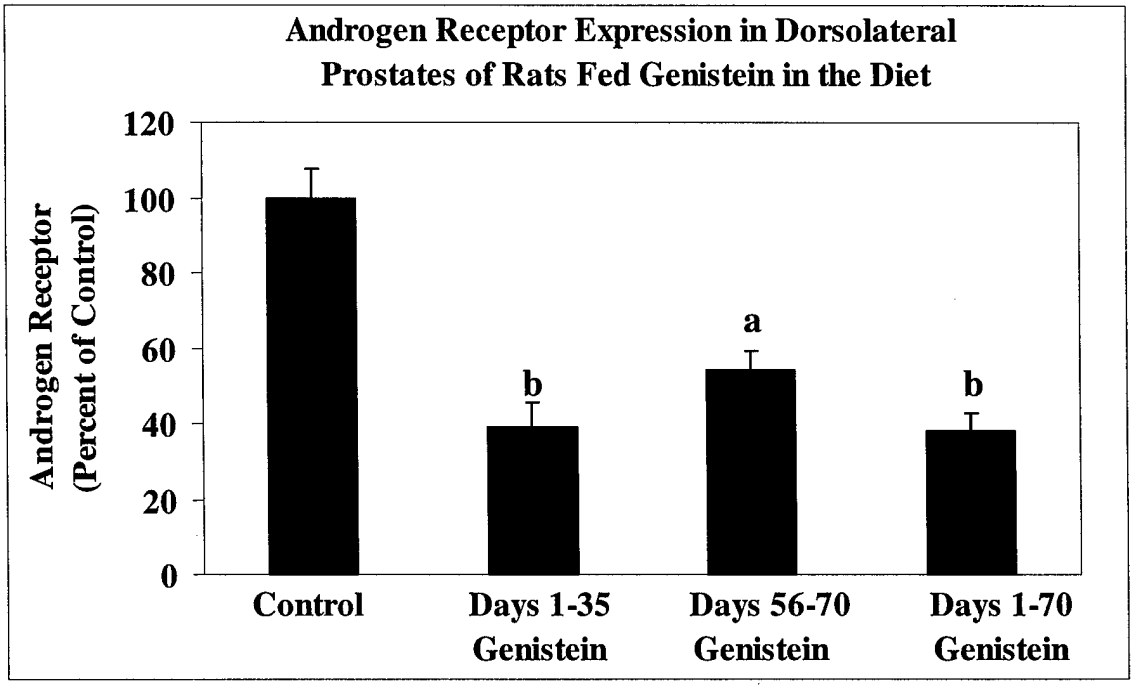

Figure 1. Dietary genistein reduces androgen receptor expression in dorsolateral prostates of rats. a = p< 0.01 and b = p < 0.001 compared to controls, respectively as determined by one-way ANOVA. Each group contained 6 samples, 2 prostates/sample).

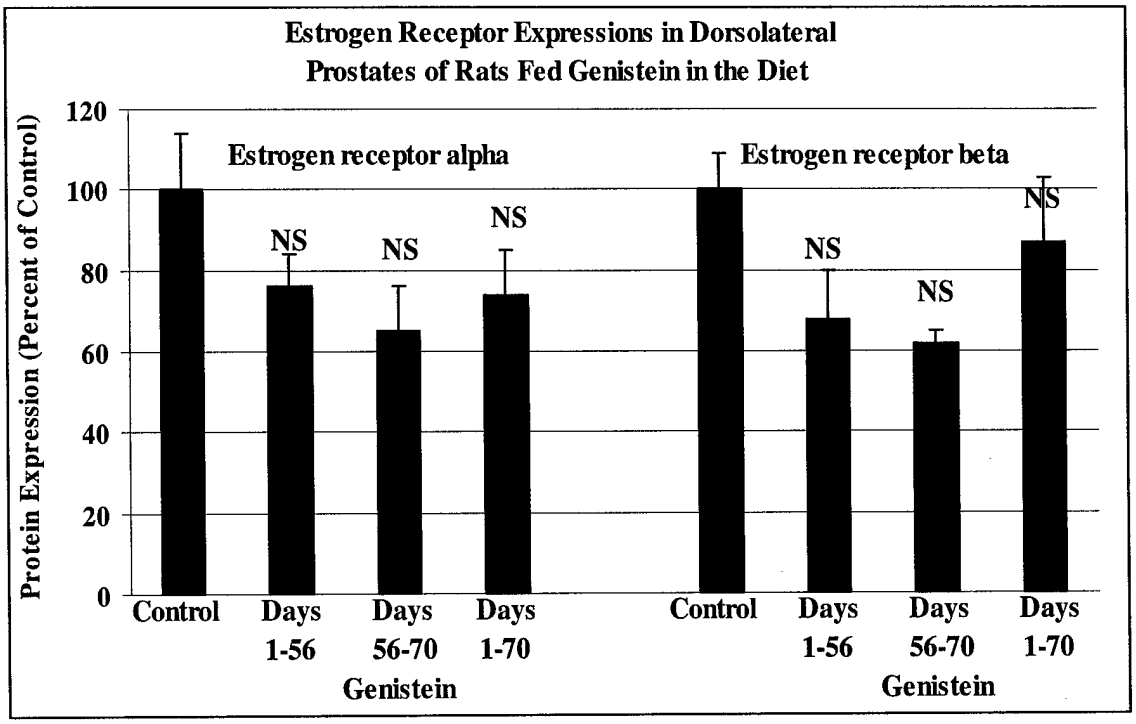

Figure 2. Estrogen receptor expression in dorsolateral prostates of rats fed genistein in the diet. NS: No significant effect compared to controls as determined by one-way ANOVA. Each group contained 6 samples, 2 prostates/sample).

cause a permanent effect on gene and protein expressions is via DNA methylation (See next Aim).

Aim 4. To investigate DNA methylation as imprinting mechanism of action. This is to be carried out during year 3. From the data in Figures 1 and 2, it is evident that the "permanent" down regulated expression of the androgen receptor could be explained on the basis of DNA methylation. In year 3, we will treat rats as described above with genistein, isolate DNA from the prostates, subject the DNA to bisulfite modification followed by PCR with methylation specific primers and gel analysis for methylation specific PCR analysis.

Key Research Accomplishments

Prepubertal, as well as adult and lifetime, exposure to genistein in the diet down regulated androgen receptor, but not estrogen receptors alpha and beta, expression in the dorsolateral prostates of rats.

Reportable Outcomes

None.

Conclusion

Down regulated androgen receptor is consistent with genistein suppressing prostate cancer development. Short term exposure to genistein having a permanent effect on androgen receptor expression is hypothesized to occur via androgen receptor promoter DNA methylation.