Repeat Dose Rabbit Vaginal Tolerance/Toxicity Study of Diindolylmethane Cream for Topical Treatment of Cervical Intraepithelial Neoplasia

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ABSTRACT
Cervical intraepithelial neoplasia (CIN) is a preneoplastic condition characterized by dysplastic changes in the cervix. Because CIN is the precursor of cervical cancer, the American Cancer Society recommends annual Papanicolaou smear screening of all women 18 to 69 years of age to detect and treat it. To prevent cervical cancer, pharmaceutical companies have developed compounds that can prevent or reverse dysplastic cervical lesions, and these agents are in clinical trials. Design of clinical trials to evaluate new cervical cancer chemopreventive agents is challenging due to the high incidence of CIN, and the need for relatively large numbers of participants. This study evaluated the systemic and local toxicity of three concentrations of DIM (2%, 4%, and 6%) applied transvaginally in the New Zealand white rabbit. Three New Zealand white rabbits were treated with 2% (placebo cream), 4% DIM, or 6% DIM for three consecutive days per week for 13 weeks. Subchronic vaginal irritation and erythema scores were significantly increased in rabbits treated with 4% DIM and 6% DIM. Plasma DIM concentrations were dose-dependent, especially at 2 hours post-dosing. A significant decline in vaginal microflora was seen in all study groups. This study evaluated the effects of DIM on vaginal microflora in the rabbit. The results of this preclinical study may be useful for the design of clinical trials for the prevention of cervical dysplasia.

INTRODUCTION
Cervical Cancer Prevention by Indoles

Most early studies of cancer chemoprevention by I3C and DIM were focused on the activity of these agents as inhibitors of experimental tumors in animal models. Early reports also suggested that I3C and DIM may have beneficial effects in the prevention of human cancers. Mechanisms of Cancer Chemoprevention by Indole Derivatives

Most clinical studies of I3C and DIM have focused on the activity of these agents as inhibitors of experimental tumors in animal models and on their clinical potential to reduce the risk of cancer in humans. Cruciferous vegetables contain several classes of chemicals whose biological activities include isothiocyanates and derivatives of I3C, and DIM have a unique set of therapeutic properties for the treatment of cancer.

NATURAL HISTORY
This naturally occurring isothiocyanate is present in cruciferous vegetables and has tumor chemoprevention properties associated with it. DIM has been identified as a high priority candidate for evaluation in human cancer chemoprevention. This study was performed to characterize the potential local and systemic toxicity that may result from subchronic intravaginal administration of DIM in the New Zealand white rabbit (Oryctolagus cuniculus).

STUDY GOALS
To determine the systemic and local toxicity of DIM applied by the intravaginal route for the treatment of cervical intraepithelial neoplasia (CIN).

MATERIALS & METHODS
Animal Selection
Rabbits weighed approximately 3 kg at the time of receipt, and were held in quarantine for approximately 3 weeks prior to the study. Three New Zealand white rabbits were used in the study, and were of similar age and weight.

STUDY DESIGN
The animals were housed in the Animal Facility at the IIT Research Institute (Willowbrook, IL). The Animal Facility was maintained in accordance with the guidelines of the American Association for Accreditation of Laboratory Animal Care, the American Veterinary Medical Association, and the Animal Welfare Act of the United States. The facility was maintained on a 14-hr light:10-hr dark cycle (lights on 0700-2100). The baseline diet for the rabbits was Certified Rabbit Chow #2031C (Harlan/Teklad, Madison, WI).

STUDY GOALS
To determine the systemic and local toxicity of DIM applied by the intravaginal route for the treatment of cervical intraepithelial neoplasia (CIN). This study was performed to characterize the potential local and systemic toxicity that may result from subchronic intravaginal administration of DIM in the New Zealand white rabbit (Oryctolagus cuniculus).

RESULTS
Morbidity
No morbidly was seen in any group during the study.

Vaginal Irritation Scoring
To determine if DIM caused systemic toxicity, tissue distribution, and/or pharmacokinetic factors associated with oral administration may limit the concentration of DIM in the target tissues.

Table 2: Vaginal Irritation Scores (Degree Scoring)

Table 3: Incidence of Synaptohistiolytic Activity of the Cervical Epithelium

CONCLUSIONS
1. Repeat dose intravaginal administration of the maximum tolerated dose (MTD) of DIM (2%, 4%, or 6%) to rabbits was well tolerated. Body weights, food consumption, and the results of clinical observations and clinical pathology evaluations in DIM treated rabbits were comparable to those in placebo treatment groups.
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