Available online at www.sciencedirect.com
ScienceDirect
Chemico-Biological Interactions 164 (2006) 68–75
Chemico-Biological Interactions
www.elsevier.com/locate/chembioint

Effects of oral dosing paradigms (gavage versus diet) on pharmacokinetics and pharmacodynamics☆

I.M. Kapetanovic[a,*], R. Krishnaraj[b], T. Martin-Jimenez[c], L. Yuan[d], R.B. van Breemen[d], A. Lyubimov[b]

[a] *Chemopreventive Agent Development Research Group, Division of Cancer Prevention, National Cancer Institute, Bethesda, MD 20892-7322, United States*
[b] *Toxicology Research Laboratory, Department of Pharmacology, University of Illinois at Chicago, Chicago, IL 60612, United States*
[c] *Department of Comparative Medicine, University of Tennessee, Knoxville, TN 37996, United States*
[d] *Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, United States*

Received 26 June 2006; received in revised form 23 August 2006; accepted 26 August 2006
Available online 1 September 2006

Abstract

In cancer chemopreventive studies, test agents are typically administered via diet, while the preclinical safety studies normally employ oral gavage dosing. Correspondence in pharmacokinetic and pharmacodynamic profiles between the two dosing approaches cannot be assumed *a priori*. Sulindac, a non-steroidal anti-inflammatory agent with potential chemopreventive activity, was used to assess effects of the two oral dosing paradigms on its pharmacokinetics and pharmacodynamics. Time-dependent concentrations of sulindac and its sulfone metabolite were determined in plasma and potential target organ, mammary gland. Prostaglandin E_2 was used as a pharmacodynamic biomarker and measured in mammary gland. An inverse linear relationship was detected between pharmacodynamic and pharmacokinetic markers, area under the curve for prostaglandin E_2 levels and sulindac sulfone concentrations, respectively, in the mammary tissue. Marked differences in pharmacokinetics and pharmacodynamics were observed after administration of sulindac by the two oral dosing paradigms. In general, oral gavage resulted in higher peak and lower trough concentrations of sulindac in plasma and mammary tissue, higher area under concentration–time curve in plasma and mammary tissue, and greater effect on prostaglandin E_2 levels than the corresponding diet dosing. This study illustrates potential pitfalls and limitations in trying to generalize based on data obtained with different oral dosing schemes and their extrapolation to potential efficacy and health risks in humans.
Published by Elsevier Ireland Ltd.

Keywords: Oral gavage; Diet dosing; Pharmacokinetics; Pharmacodynamics; Sulindac; PGE2

1. Introduction

Extrapolation from animal data to human dose selection is complicated and can be further confounded by different dosing paradigms. Preclinical safety pharmacology studies normally employ oral gavage method of test agent administration in standard rodent protocols. On the other hand, efficacy studies frequently

☆ Presented at the 2006 SOT meeting in San Diego, CA.

* Corresponding author at: Chemopreventive Agent Development Research Group, Division of Cancer Prevention, National Cancer Institute, 6130 Executive Blvd, Suite 2117, MSC 7322, Bethesda, MD 20892-7322, United States. Tel.: +1 301 435 5011; fax: +1 301 402 0553.
E-mail address: kapetani@mail.nih.gov (I.M. Kapetanovic).

0009-2797/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.cbi.2006.08.019

administer test agents via feed. Test agents are typically administered to rodents via diet in cancer chemopreventive studies [1]. Diet is the most prevalent dosing method in chemopreventive studies for number of reasons, including ability and ease of dosing large number of animals, availability of historical data, and because many candidate chemopreventive agents are naturally found in the diet. However, the two approaches for administering the test agent can be expected to yield differences in pharmacokinetic and pharmacodynamic profiles. The present study was undertaken to systematically assess the two oral dosing paradigms, gavage and diet, on disposition of a test agent in rats and its effect on a biological biomarker of exposure.

Sulindac was selected in the present study because it has potential applications in cancer chemoprevention, a reasonable half-life in rats, a reasonable pharmacodynamic biomarker, and metabolites that can also be studied. Sulindac belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDS). NSAIDS have shown promise as chemopreventive agents and their action is believed to be at least in part by inhibition of cyclooxygenase 2 (COX2) activity [2,3]. The mammary gland is one of several potential target organs for chemopreventive activity of NSAIDS, including sulindac [4–6]. Cyclooxygenase enzymes (COX-1, COX-2, and COX-3) convert arachidonic acid to prostaglandins, prostacyclins, thromboxanes, and other hydroxy fatty acids. Prostaglandin E_2 (PGE_2) has tumor growth-promoting activity and inhibition of its formation has been commonly used as a measure of pharmacodynamic effect [7,8], including that in mammary tissue [7,9].

2. Materials and methods

2.1. Chemicals

Sulindac (2-[(3*Z*)-6-fluoro-2-methyl-3-[(4-methylsulfinylphenyl)methylidene]inden-1-yl]acetic acid) was obtained from Spectrum Chemical Manufacturing Corp. (Gardena, CA). It was identified by GC–MS. The purity was determined by HPLC prior to initiation of dosing and after completion of the in-life phase, and was stable under the storage conditions. Sulindac sulfone was from LKT Laboratories (St. Paul, MN).

2.2. Animals

Female Crl:CD (Sprague–Dawley) virus antibody free (VAF) rats were obtained from Charles River Breeding Laboratories (Kingston, NY). The animals were approximately 6–7 weeks old upon receipt and weighed 153–220 g at dosing initiation. Animals were singly housed in polycarbonate cages with Anderson bedo'cobs® bedding (Heinold, Kankakee, IL) in a temperature (64-79 °F) and humidity (30–70%) controlled room on a 14 h light/10 h dark cycle. Certified Rodent Chow No. 5002 (PMI Feeds, Inc., St. Louis, MO) and local drinking water were provided from arrival until termination. The study was approved by UIC Animal Care Committee.

2.3. Preparation of sulindac containing diet

Sulindac was mixed with the Certified Rodent Chow No. 5002 diet to provide a nominal exposure of 80 and 200 ppm of sulindac in the diet. Diets were prepared weekly and stored at room temperature. Concentration of sulindac in representative batches was analyzed prior to use and were stable for at least 1 week. Only diets within 10% of target concentrations were used. Although the nominal diet dose levels were 8 and 20 mg/kg/day, the average calculated diet doses (±S.E.M.) were 7.4 mg/kg/day (±0.055) and 6.7 mg/kg/day (±0.13) in weeks 1 and 2, respectively, for the nominal 8 mg/kg/day diet dose group. The calculated diet doses (±S.E.M.) were 16.6 mg/kg/day (±0.094) and 16.2 mg/kg/day (±0.18) in weeks 1 and 2, respectively, for the nominal 20 mg/kg/day diet dose group.

2.4. Experimental design

Animals (five per dose per time point) were randomly assigned based on body weights to four treatment groups: 8 mg/kg/day oral gavage, 20 mg/kg oral gavage, 80 ppm diet and 200 ppm diet. For animals in the oral gavage groups, the test article was administered once daily by gavage at a dosing volume of 5 ml/kg/day in 1% carboxymethylcellulose. For animals assigned to dietary administration groups, the diet was supplemented with sulindac to achieve target concentrations and animals had an *ad libitum* access to food. All animals were euthanized by CO_2 and sacrificed on the 15th day at 0, 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 24 h after the last gavage dose. Animals in the corresponding diet groups were sacrificed in parallel at the same time points. At the time of sacrifice, blood and mammary gland (inguinal regions from left and right side) were collected from each animal. Blood samples were collected from vena cava in heparin tubes, plasma harvested using standard procedures and stored at approximately −80 °C.