BIOCHEMICAL PHARMACOLOGY 73 (2007) 1280–1287

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Efficacy of increasing the therapeutic index of irinotecan, plasma and tissue selenium concentrations is methylselenocysteine dose dependent

Rami G. Azrak [a,*], *Shousong Cao* [a], *Lakshmi Pendyala* [b], *Farukh A. Durrani* [a], *Marwan Fakih* [b], *Gerald F. Combs Jr.* [c], *Joshua Prey* [b], *Patrick F. Smith* [b], *Youcef M. Rustum* [a]

[a] *Department of Cancer Biology, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY, United States*
[b] *Department of Medicine, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY, United States*
[c] *Grand Forks Human Nutrition Research Center, USDA-ARS, Grand Forks, ND, United States*

ARTICLE INFO

Article history:
Received 10 November 2006
Accepted 18 December 2006

Keywords:
Methylselenocysteine
Selenium
FaDu
A253
Irinotecan

ABSTRACT

This study was designed to understand the basis for the efficacy of methylselenocysteine (MSC) in increasing the therapeutic index of irinotecan against human tumor xenografts. Nude mice bearing human head and neck squamous cells carcinoma xenografts (FaDu and A253) were treated orally with different doses of MSC and irinotecan. Plasma, tumor and normal tissue samples were collected at different times after MSC treatments and were analyzed for selenium (Se) concentration using electrothermal atomic absorption spectrophotometry. MSC is highly effective in modulating the therapeutic index of irinotecan. Enhanced irinotecan efficacy was greater in FaDu tumors (100% CR) than in A253 tumors (60% CR), and depended on MSC dose with a minimum effective dose of 0.01 mg/d × 28. The highest plasma Se concentration was achieved 1 h after a single dose and 28 d after daily treatments of MSC. The ability of FaDu tumors to retain Se was significantly better than A253 tumors, and the highest Se concentration in normal tissue was achieved in the liver. Peak plasma and tissue Se concentrations were functions of the dose and duration of MSC treatment. The MSC-dependent increase in Se level in normal tissues may contribute to the protective effect against irinotecan toxicity observed in those tissues. Intratumoral total Se concentration was not found to be predictive of the combination therapy response rates. There is a critical need to develop a method to measure the active metabolite of MSC, rather than total Se.

Published by Elsevier Inc.

1. Introduction

Selenium (Se) is an essential trace element in human and animal nutrition. A significant body of animal model and epidemiological work indicates that Se also can reduce cancer risk [1–3]. Several mechanisms have been proposed for this anticarcinogenic action: regulation of p53 by the Ref1 dependent redox mechanism [4,5]; induction of apoptosis associated with increased phosphorylation of p53 MAPK and dephosphorylation of Akt, ERK-1 and ERK-2 [6–8]; and

* *Corresponding author*. Tel.: +1 716 845 4940; fax: +1 716 845 4928.
E-mail address: Azrak@roswellpark.org (R.G. Azrak).

Abbreviations: MSC, methylselenocysteine; Se, selenium; MTD, maximum tolerated dose; CR, cure rates; i.v., intravenously; s.c., subcutaneously; QC, quality control; AUC, area under the concentration time curve

0006-2952/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.bcp.2006.12.020

antiangiogenic activity by inhibiting the expression of vascular endothelial growth factors (VEGFs) [9], among others. Although most preclinical chemoprevention studies have used inorganic sodium selenite, the most informative human trial [10] used a Se-enriched yeast. Species of Se found in yeasts are reported to be dependent on the manufacturers and methods of detection. Although yeast Se is thought to be predominantly in a form of selenomethionine, other forms of yeast Se have been reported, such as selenocysteine, methylselenocysteine and unidentified selenium species [2,11–13]. In the selenium intervention trial by Clark et al. administration of 200 μg of yeast Se was shown to reduce the incidence of several types of cancers [10,14]. These results are consistent with most epidemiological studies showing Se status to be inversely associated with cancer risk [1,15,16]. Based on these data, the selenium and vitamin E cancer prevention trial (SELECT) was initiated to determine the effects of Se and vitamin E in preventing prostate cancer [17]. L-Selenomethionine is one of the two intervention agents used in this trial.

Ip [2] has proposed that the anticarcinogenic actions of Se involve the formation of methylselenol (CH_3SeH) as the active metabolite. Methylselenocysteine (MSC), a stable, water-soluble Se-compound that is quantitatively absorbed when taken orally [18], is then hydrolyzed by a β-lyase to yield methylselenol [2,10,19].

Previously, Azrak et al. [20] characterized untreated controls of A253 and FaDu human xenografts of the head and neck squamous cell carcinomas (HNSCC) using immunohistochemistry. A253 tumors are well-differentiated with an average doubling time of ~3.25 d. FaDu tumors are poorly differentiated with an average doubling time of ~2.9 d. Cao et al. [21] reported that oral administration of MSC at the maximum tolerated dose (MTD, 0.2 mg/mouse) on a daily × 28 schedule (0.2 mg/d × 28), in combination with the weekly intravenous (i.v.) schedule of irinotecan ($C_{33}H_{38}N_4O_6{\cdot}HCl{\cdot}3H_2O$) (100 mg/kg week^{-1} × 4, MTD), increased the cure rate from 30% (irinotecan alone) to 100% in FaDu and from 10 to 60% in A253 xenografts. Oral pretreatment with MSC daily for 7 d prior to initiation of irinotecan treatment was essential for optimal therapeutic synergy.

In this study, we determined that the enhanced antitumor activities of irinotecan and protections from its induced toxicity are MSC dose dependent. The minimum MSC dose that is needed to achieve 100% cure rates of irinotecan in FaDu xenografts was identified. In addition, we evaluated the pharmacokinetic profiles of Se in the plasma and select tissues after treatment with various doses of MSC, demonstrated that FaDu xenografts are capable of retaining Se more effectively than A253 xenografts, and determined whether Se levels are predictive of the observed therapeutic synergy of MSC and irinotecan.

2. Materials and methods

2.1. Mice

Eight to 12-week-old, female athymic nude mice (nu/nu, body weight 20–25 g) were obtained from Harlan Sprague–Dawley Inc. (Indianapolis, IN). The mice were housed, five mice/cage, under specific pathogen-free conditions with water and food provided *ad libitum*, according to a protocol approved by the Institute Animal Care and Use Committee at Roswell Park Cancer Institute.

2.2. Tumors

Two human head and neck tumor xenografts (FaDu and A253) were used. Xenografts were initially established by implanting subcutaneously (s.c.) 10^6 cultured cells and passed several generations by transplanting ~50 mg non-necrotic tumor tissue before irinotecan treatment, which began about 1 week after implantation, when the tumors were 100–200 mg in size.

2.3. Drugs and treatments

Irinotecan was purchased from Pharmacia (Kalamazoo, MI), as a ready-to-use clinical formulation solution in 5 ml vials containing 100 mg of drug (20 mg/ml). Irinotecan was administered (100 and 200 mg/kg week^{-1} × 4) by i.v. injection via the tail vein of animals. Methylselenocysteine hydrochloride ($C_4H_9NO_2Se{\cdot}HCl$) was purchased from Sigma (St. Louis, MO), as a powder in 100 mg/vial and dissolved in 0.9% NaCl at a final concentration of 1 mg/ml. It was administered daily by oral gavages (7 d prior to irinotecan administration and continued for 28 d) at different doses: 0.001, 0.005, 0.01, 0.05, 0.1, 0.2 (MTD) mg/d.

Treatments were administered to 10 mice in each group per experiment and every experiment was repeated twice. Cure rates (CR) were defined as no detectable tumor at the site of transplant for up to 3 months after termination of treatment. Plasma samples were collected: (a) 1, 2, 4, 8, 12, 16, and 24 h after treatment with a single dose of MSC at 0.2 mg/d; (b) 2 h after treatment with MSC (0.2 mg/d) for 7, 14, 21 and 28 d; (c) 2 h after 7 d treatment with different doses of MSC (0.005, 0.01, 0.05, 0.1, 0.2 mg/d × 7). Tissue samples were collected at 2 h after 7 d treatment with various doses of MSC (0.005, 0.01, 0.05, and 0.2 mg/d × 7).

2.4. Total selenium measurement analysis

Total Se in plasma and tissues was measured by electrothermal atomic absorption spectrophotometry, using a PC-based ZL4100 Atomic Absorption Spectrophotometer (Perkin-Elmer) equipped with autosampler or automated Zeeman-effect background correction (Spectra AA-600, Varian Instruments, Walnut Creek, CA). Tissues were homogenized in 0.25% triton X-100 (10 ml diluent/1 g tissue weight) using a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, NY). Standard curves ranging from 40 to 800 ng/ml were prepared using the selenium atomic absorption spectroscopy analytical standard (Perkin-Elmer) or L-selenomethionine, and calibrated against standardized human plasma (Seronorm, Billingstad, Norway). Plasma and tissue homogenates were diluted 1:5 in a diluent consisting of 0.2% nitric acid, 0.1% Triton™ X-100, 1% $Pd(NO_3)_2$ and 0.1% $Mg(NO_3)_2$ prior to a 20 μl injection. The matrix modifiers $Pd(NO_3)_2$ and $Mg(NO_3)_2$ decreased the volatility of Se and prevented its loss during thermal pretreatment. They also increased the volatility of matrix components and promoted their removal before