Are We Systematically Under-Dosing Patients With Fluorouracil?

Introduction

Fluorouracil (FU) analogs are widely used but incompletely effective for cancer treatment. Drug treatment failure may be unnecessarily high as a result of suboptimal dosing, yet finding the correct dose is difficult. Individualized dose calculation using blood monitoring was reported to achieve a target plasma level, overcoming variable plasma FU levels among patients.^{1,2} It might be yet more informative to assay the end effects on tissues, but this had not been done in patients.

Thymidylate synthase (TS) is covalently modified after FU, inhibiting its function. This adducted TS (TS-F) protein manifests as a slower species in protein gels or as a neoepitope recognized by a specific antibody (FTS; EMD Millipore, Billerica, MA).^{3,4} TS is detectable by immunoblotting from human tumors and proliferative normal tissues such as duodenal mucosa.⁵ TS-F is stable, being observed in cultured cells, xenografts, and normal tissues even weeks after a single short exposure of FU.^{4,5} The ratio of TS-F to TS and dose relationships were explored in FU-treated cells, murine tissues, and xenografts.^{4,6}

We present here the clinical quantitative detection of FU activity (the ratio) in tissues from two patients receiving the FU analog capecitabine by neoadjuvant dosing.

Case Reports

Two patients with obstructive jaundice entered this IRB-approved study. Informed and written consent was obtained. Patients received 1000 mg/m² of capecitabine orally 2 hours and 12 hours before incision for pancreaticoduodenectomy. Patient 1 (P1) was a 77-year-old woman. A 4-mm well-differentiated duodenal adenocar-

cinoma was resected. Patient 2 (P2) was a 63-year-old man. A 2-cm well-differentiated adenocarcinoma in the pancreas was removed. Their normal duodenal margins were flash-frozen. A similar sample from an untreated patient (P0) was acquired from another IRB-approved study. Tissues were processed as described.⁵ AAV-293 cells were treated with FU (10 μ mol/L) or vehicle control for 24 hours and processed as controls.

Proteins were separated in 10% Bis-Tris gels (Life Technologies, Carlsbad, CA), transferred to polyvinylidene difluoride membrane, and immunoblotted with antibodies (rat anti-adducted TS-specific antibody, FTS⁴; rabbit anti-TS [Epitomics, Burlingame, CA]; and anti- α -tubulin [Santa Cruz, Dallas, TX]). Membranes were imaged by chemiluminescence (ThermoScientific, Waltham, MA).

Anti-TS antibody recognized a single protein band in untreated cells and two bands, TS-F (Fig 1, black arrow) and unaffected TS (white arrow), in FU-treated cells. FTS antibody recognized the TS-F band only in FU-treated cells. Anti-TS recognized a single band in the untreated duodenum (Fig 1, P0) and two bands, TS-F and unaffected TS, in the duodenum of treated patients (P1 and P2). In both, the doses of FU caused covalent inactivation of much, but not all, of the TS protein as observed on the first day postdosing in duodenal mucosa. The ratio of TS-F to unaffected TS was higher in patient 2 than in patient 1, a difference seen in replication blots when comparing graded exposures of membranes. FTS antibody recognized a single band of TS-F in the duodenum only from treated patients.

Discussion

In both of two patients treated conventionally with capecitabine, we found incomplete clinical conversion of TS to its inactive fluoropyrimidine-adducted form. Almost 100% experimental conversion is readily achieved in most non-microsatellite unstable cell lines in vitro and in murine tissues in vivo.⁵ Our conventionally dosed patients appeared to have subtotal conversion and thus perhaps a

Fig 1.

suboptimal drug effect on bodily TS protein. Although TS is not the only relevant target of FU,⁴ one may speculate that conventional schedules may systematically underdose many patients. Testing acutely treated patients, as done here, may provide advantages owing to the stability and possible accumulation of inactive TS-F⁵ with daily dosing. Difficulties in observing a reduction in unmodified TS in blood mononuclear cells among capecitabine-treated patients was reported.⁷ We suggest that the tissue measurement of TS conversion could be evaluated for any clinical predictive value in therapeutic effectiveness and as an algorithmic approach to calculate proper dosing of FU and capecitabine response in patients, conceivably in the future using a minimally invasive mucosal biopsy or swab.

Kalpesh Patel and Christine A. Iacobuzio-Donahue

Johns Hopkins Medical Institutions, Baltimore, MD

Paul E. Gormley

St Agnes Cancer Institute, Baltimore, MD

Scott E. Kern

Johns Hopkins Medical Institutions, Baltimore, MD

Steven C. Cunningham

St Agnes Cancer Institute, Baltimore, MD

ACKNOWLEDGMENT

We thank Ralph Hruban, MD, William Hicken, MD, and Jeffery Seibel, MD, for their histopathology interpretations and tissue procurement, and Carole Miller, MD, and Enser Cole, MD, for their support and advice on chemotherapy. This work was supported National Cancer Institute grant No. CA62924 and by the Everett and Marjorie Kovler Professorship in Pancreas Cancer Research. S.E.K. and S.C.C. contributed equally and share senior authorship.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a

financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

Employment or Leadership Position: None **Consultant or Advisory**

Role: None **Stock Ownership:** None **Honoraria:** None **Research**

Funding: None **Expert Testimony:** None **Patents, Licenses, or**

Royalties: Kalpesh Patel, licensed FTS antibody to Millipore; Scott E.

Kern, licensed FTS antibody to Millipore **Other Remuneration:** None

REFERENCES

1. Gamelin E, Delva R, Jacob J, et al: Individual fluorouracil dose adjustment based on pharmacokinetic follow-up compared with conventional dosage: Results of a multicenter randomized trial of patients with metastatic colorectal cancer. *J Clin Oncol* 26:2099-2105, 2008
2. Kline CL, Sheikh HS, Scicchitano A, et al: Preliminary observations indicate variable patterns of plasma 5-fluorouracil (5-FU) levels during dose optimization of infusional 5-FU in colorectal cancer patients. *Cancer Biol Ther* 12:557-568, 2011
3. Johnston PG, Liang CM, Henry S, et al: Production and characterization of monoclonal antibodies that localize human thymidylate synthase in the cytoplasm of human cells and tissue. *Cancer Res* 51:6668-6676, 1991
4. Patel K, Yerram SR, Azad NA, et al: A thymidylate synthase ternary complex-specific antibody, FTS, permits functional monitoring of fluoropyrimidines dosing. *Oncotarget* 3:678-685, 2012
5. Brody JR, Gallmeier E, Yoshimura K, et al: A proposed clinical test for monitoring fluoropyrimidine therapy: Detection and stability of thymidylate synthase ternary complexes. *Cancer Biol Ther* 5:923-927, 2006
6. Drake JC, Allegra CJ, Johnston PG: Immunological quantitation of thymidylate synthase-FdUMP-5,10-methylenetetrahydrofolate ternary complex with the monoclonal antibody TS 106. *Anticancer Drugs* 4:431-435, 1993
7. Perotti A, Locatelli A, Sessa C, et al: Phase IB study of the mTOR inhibitor ridaforolimus with capecitabine. *J Clin Oncol* 28:4554-4561, 2010

DOL: 10.1200/JCO.2013.49.5044; published online ahead of print at www.jco.org on March 10, 2014