Pharmacodynamic Modelling of Anti-Proliferative Effects of Tetraiodothyroacetic Acid (Tetrac) on Human Cancer Cells

Contact:
clandersdorfer@
ordwayresearch.org

C.B. Landersdorfer[1], D. London[1], R. Meng[1], C. Lin[1], S. Lin[1], C.-U. Lim[1], L. Queimado[2], S.A. Mousa[3], G.L. Drusano[4], A. Louie[4], F.B. Davis[1], H.-Y. Lin[1], P.J. Davis[1].

[1]The Signal Transduction Laboratory, Ordway Research Institute, Albany, NY; [2]The Department of Otorhinolaryngology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK; [3]The Pharmaceutical Research Institute at Albany College of Pharmacy, Rensselaer, NY; [4]The Emerging Infections and Pharmacodynamics Laboratory, Ordway Research Institute, Albany, NY.

Introduction

Tetraiodothyroacetic acid (tetrac), a deaminated analogue of L-thyroxine (T_4), competes with T_4 to bind to the integrin αvβ3 receptor on the cell surface and induces apoptosis and anti-proliferation in several kinds of cancer cells. We sought to develop a PD model to characterize these effects.

Objective

Our aim was to develop a mechanism-based pharmacodynamic model that characterizes the action of tetrac on human cancer cells in a newly developed perfusion bellows cell culture system.

Methods

Perfusion bellows cell culture system

Each of the newly developed perfusion bellows cell culture systems consists of a 500 mL bottle which contains both cell culture medium and specially treated flakes. Cells are introduced into the bottles, and then attach to and grow on the flakes. Through moving bellows and porous membranes the level of the medium in the bottle changes periodically and consequently the cells are alternately submerged in the culture medium and exposed to 95% air / 5% CO_2. In addition, the system is constantly perfused by fresh culture medium (one complete change / 24 h, depending on flow rate).

This system allows for maximized nutrient uptake and oxygen transfer for the cells and thereby optimized growth conditions.

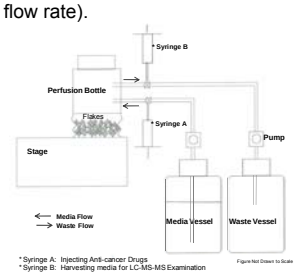

Figure 1: Scheme of perfusion bellows cell culture system

Cell culture studies

Study 1: Estrogen receptor negative human breast cancer MDA-MB-231 cells were seeded in the perfusion bellows cell culture system containing DMEM supplemented with 1% fetal bovine serum (FBS). The cells were treated continuously with seven different concentrations of tetrac ranging from 10^{-8} M to 10^{-5} M, or with control medium for 19 days.

Study 2: In additional studies human glioblastoma U87MG cells grown in MEM supplemented with 1% FBS were also treated with three different concentrations of tetrac in the perfusion bellows cell culture system.

In studies 1 and 2 flakes were collected every one or two days and cells harvested from the flakes for counting.

Study 3: Human colon cancer Colo-205 cells were grown in RPMI medium containing 10% FBS in T75 flasks and treated with three different concentrations of tetrac or drug-free medium for 18 days. Media with or without tetrac were refreshed daily. Total cell counts were performed at the start of treatment and every one or two days thereafter.

Pharmacodynamic modeling

All treatment arms within each experiment were modeled simultaneously in NONMEM VI using a pooled approach. Simulation-estimation experiments were run with 50 datasets per study assuming a rich sampling schedule and an additive error on $\log_{10}$-scale of 0.1 (studies 1 and 2) or 0.05 (study 3) utilizing both NONMEM and S-ADAPT (MC-PEM algorithm).

Results

Figure 2: Model diagram

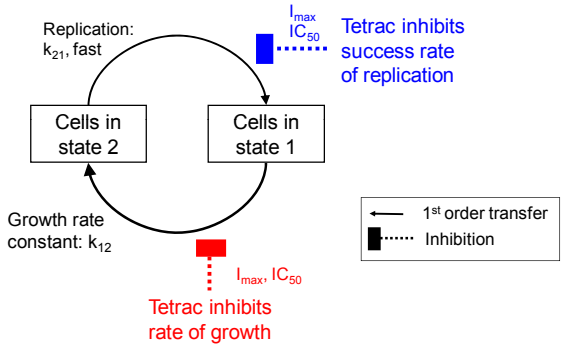

Figure 2 shows the diagram of a mechanism-based model which was adapted from [1] to describe the proliferation of cancer cells and inhibition of proliferation by tetrac. This model assumes two populations of cells in different states of the cell cycle: cells that are preparing for replication (state 1) and cells that are immediately "pre-replication" (state 2). Cells transition from state 1 to state 2 by a first-order growth rate constant, while replication from state 2 to state 1 is assumed to be fast.

Figure 3: Effect of tetrac on human breast cancer (MDA-MB) cells

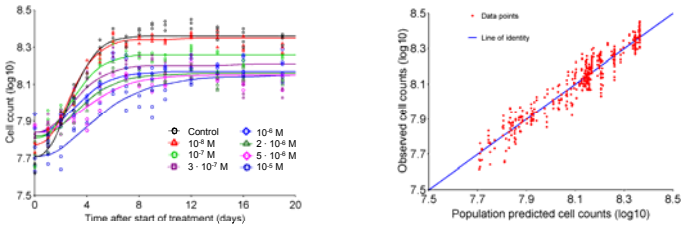

Figure 4: Effect of tetrac on Colo-205 and U87MG cells

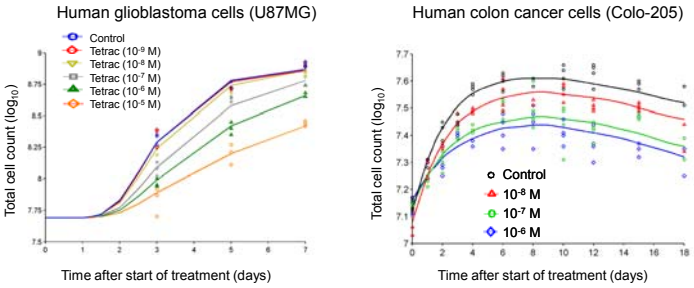

Table 1: Parameter estimates and bias (MC-PEM / FOCE) for tetrac effects

	Effect on rate of growth		Effect on success of replication	
Cell line	I_{max}	IC_{50} (μM)	I_{max}	IC_{50} (μM)
MDA-MB	0.85 +3% / +0.6%	5.1 +9% / -0.4%	0.20 -2% / +0.7%	0.087 +6% / +5%
U87MG	0.57 -5% / -3%	0.047 +26% / +19%	0.92 +0.01% / -3%	47.4 -0.4% / -4%
Colo-205	effect not included		0.17 +0.1% / +0.3%	0.020 -5% / -6%

Conclusions

The developed mechanism-based model successfully described the observed net effects of tetrac on cells derived from glioblastoma and colon cancer and from human breast cancer. The model includes two effects of tetrac on different parts of the cell cycle which can be distinguished.

Modeling suggests that the effect of tetrac on the probability of successful replication is most important for the colon cancer cells grown in flasks, whereas both effects are necessary to describe the effects of tetrac on breast cancer and glioblastoma cells in the perfusion bellows system.

Future perspectives

By adjusting the flow rate of the medium and the dosing schedule, drug concentration-time profiles as expected in human or animal studies can be simulated in the perfusion bellows cell culture system. Thereby the effects on cancer cells of changing drug concentrations as anticipated *in vivo* may be observed in the *in vitro* system.

In combination with pharmacodynamic modeling and by including information about the expected pharmacokinetics of a drug, the perfusion bellows cell culture system allows one to study the dose-response relationship of anti-neoplastic agents over a very wide concentration range *in vitro*, and can support translation from *in vitro* to animal models and human clinical trials.

Reference
[1] Bulitta J.B. et al. (2009) Antimicrob Agents Chemother;53:46-56.