MHR

A. G. PAYNE [2004] MED HYPOTHESES RES 1: 247-252.

EFFECTING ONCOLYSIS BY DEPLETING INTRACELLULAR GLUTATHIONE, BOOSTING OXIDATIVE STRESS, AND REDUCING IGF-I

ANTHONY G. PAYNE*

STEENBLOCK RESEARCH INSTITUTE, SAN CLEMENTE, CA 92673, USA.

HYPOTHESIS

ABSTRACT. THE REGIMEN CONTAINED IN THIS PAPER exploits critical pathways in the life-sustaining biology of tumor cell types in such a way that would make oncolysis more probable. This approach includes: (i) dietary methionine restriction and other means to lower intracellular glutathione with the specific goal of compromising intracellular oxidative defenses, (ii) iron loading and liberation of ferritin iron followed by measures geared to generate lethal free radical cascades, and (iii) dietary and oral agent intervention oriented towards the reduction of tumor cell-modulating IGF-I levels.

*ADDRESS ALL CORRESPONDENCE TO: DR. ANTHONY G. PAYNE, PH.D., N.M.D., M.D. (HONORARY), STEENBLOCK RESEARCH INSTITUTE, 1064 CALLE NEGOCIO #B, SAN CLEMENTE, CA 92673 USA.
PHONE: 949-248-7034; FAX: 949-388-3441.

• MEDICAL HYPOTHESES AND RESEARCH • THE JOURNAL FOR INNOVATIVE IDEAS IN BIOMEDICAL RESEARCH •

1. INTRODUCTION

The thrust of many standard oncolytic approaches involves therapeutic measures that tax the oxidative defenses of tumor cells while concomitantly generating free radicals that, in concert with oxygen, overwhelms this defense. Radiotherapy, for example, generates free radicals in nuclear DNA that interact with oxygen to produce genetic damage affected cells often cannot surmount.

Less traditional though promising methods of lethally compromising tumor cells by depleting and then overwhelming their oxidative defenses are being explored. Many of these achieve this and by dietary manipulation of specific metabolic pathways, use of botanic compounds or derivatives, and such. These often novel means of exploiting tumor cell vulnerability have been woven into regimens that have shown merit, insofar as many patients with disseminated malignancy and an almost certain terminal outcome achieved long-term remission after systematic treatment by using these metabolic oncolytic methods [1]. In this paper many of these tentatively successful elements in metabolic oncology have been married to ideas and modalities that complement their activity and intended effects. The end result is a regimen that should conceivably make tumor cell lysis more probable.

2. COMPONENTS OF THE METABOLIC SOLID TUMOR ONCOLYSIS REGIMEN (MSTOR)

The thrust of this regimen is to deplete glutathione in tumor cells, concomitant with measures geared to increase ferritin iron mediated oxidative stress, deplete ATP, and reduce the level of cell-modulating Insulin Growth Factor-1 (IGF-1). This approach complements that of many standard cancer treatments, but may be suitable as a stand-alone (heroic measures) technique. Various components of the MSTO regimen are described below.

2.1. DEPLETION OF GLUTATHIONE AND ATP

EXTREMELY LOW METHIONINE DIET. Many cancer cell lines have absolute requirements for methionine, while normal cells are relatively unaffected by methionine restriction. Studies done using animal models bearing human tumor xenographs indicate that dietary methionine restriction inhibits (tumor) growth and enhances apoptosis by depleting the ATP pool and glutathione content [2] with almost no discernable ill effects on normal tissues. This was underscored in a phase I clinical trial (Baylor College of Medicine) in which eight patients were placed on a commercially available methionine-free food and evinced only one side effect – modest weight loss (0.5% of body mass index) [3].

In the context of the regimen proposed herein, methionine restriction should be effected by use of readily available commercially canned liquid solutions, along with consumption of foods that are low in methionine. Patient intake of potatoes and vegetables plus flax seed oil is to be encouraged (in moderation), as these not only provide low levels of methionine but also helps lower cell proliferation and apoptosis modulating IGF-I (See Decreasing IGF-I section below).

THYROID. Thyroid hormone should contribute to oncolysis by: (1) increasing hepatic removal and degradation of cortisol, which brings about plasma reductions of same; and (2) stimulating ATPase activity (so as to "waste" ATP).

The lipolytic activity of thyroid hormone should be offset by the anti-lipolytic effect of insulin. (Insulin is featured in the section titled Adjuvant/adjunctive measure below)

THEANINE. Theanine is an amino acid native to green tea. Various in vitro and animal studies indicate that theanine has a tumor-specific effect on glutamate transporters located in the cell membrane [4]. Specifically, theanine binds to tumor cell glutamate transporters, thus interfering with the uptake of glutamate and the subsequent synthesis of glutathione [5]. This property suggests that oral dosing with theanine tablets or such will ably contribute to the goal of lowering tumor cell intracellular glutathione levels.

2.2. INCREASING OXIDATIVE STRESS BY EXPLOITING FERRITIN IRON

2.2.1. HYPERTHEMIA, IRON LOADING, USE OF ASCORBIC ACID, AND OTHER MEASURES

HYPERTHER-MIA THERAPY OR THERMO-RADIOTHERAPY. Localized treatment if a well-

circumscribed tumor is involved, wider scope or whole body if disseminated. The therapeutic objective is to increase tumor vascular permeability to ferritin, something hyperthermia and thermoradiotherapy has been shown to do in animal studies carried out in Japan [6].

One compelling variation of hyperthermia therapy worth considering is ferritin-mediated electromagnetic hyperthermia. This approach was ably explored in a recent study [7]. The authors contend that an alternating magnetic field no greater than ~ 100 KHz (kilohertz) should induce heating of intracellular ferritin sufficient to lyse tumor cells without adversely effecting normal tissues and cells. The iron core in ferritin is strongly paramagnetic and thus can be exploited to produce heat via the Brown and Neel effects (respectively). Since ferritin is often found at higher levels in neoplastic cells than normal ones, this makes achieving hyperthermia by way of an externally applied high frequency magnetic field very probable.

Japanese, German, and other researchers have published many papers indicating that intracellular hyperthermia sufficent to achieve cell lysis is possible employing magnetite cationic liposomes and other 'magnetic fluids.' [8,9]. The ferritin mediated approach, while different from the aforementioned, retains many features in common and should be explored in the laboratory and in well controlled clinical trials.

In 2001, I suggested the idea of exploiting iron phthalocyanines exposed to an alternating magnetic field to achieve sufficient intracellular hyperthermia to lyse tumor cells. The fact that this compound tends to be retained by tumors and is essentially non-toxic underscores its potential for oncolytic utility. Furthermore, the use of iron phthalocyanines will provide a supply of iron to tumor cells that can be exploited to generate cell lysing free radicals (discussed below).

IRON LOADING. The goal is to maximize intracellular iron levels in tumor cells. In the study cited above [1], vascular permeability to ferritin occurred at one day following hyperthermia and three days following thermoradiotherapy. It follows that treatment to enhance intracellular iron levels in patients should be done in accordance with these findings.

Candidate forms of iron can include but is not limited to ferrous sulfate, ferrous citrate, ferrous gluconate, iron-rich phthalocyanines, etc.

Supplemental ascorbate should be utilized to boost dietary absorption of iron [10,11]. Following the iron-loading phase, patients should discontinue use of vitamin C and all other antioxidants as use of these will scavenge the very free radicals needed to achieve oncolysis.

Considering contraindications, it should be noted that the use of drugs or ingestion of dietary substances may hinder or inhibit iron absorption, transport or utilization, e.g., plant phytates and tannins (e.g., tea, cereals, and grains), desferrioxamine, EDTA, manganese, zinc, ACE inhibitors, levodopa, tetracycline, and others.

2.2.2. ADJUVANT/ADJUNCTIVE MEASURE

INSULIN. The use of exogenous insulin would boost the uptake of iron by transferrin and/or possibly potentiate transferrin receptor function. This is suggested by both in vitro and in vivo studies [12,13]. If chemotherapeutic drugs are employed, insulin should increase effectiveness while simultaneously reducing the dosage needed to achieve oncolysis. This is suggested by numerous published studies, as well as a wealth of case history data.

VANADYL. Vanadyl has been shown to release iron from ferritin in a dose dependent fashion, which makes it available for oxidative reactions that generate cell lysing free radicals [14]. Interestingly, glutathione or vanadate added in relative excess to the vanadyl inhibited iron release upwards of 45% [15]. It logically follows that vanadyl introduced to a low glutathione, iron-rich tumor cell microenvironment of the sort the MSTO regimen facilitates should be able to liberate iron for use in achieving iron-catalyzed cell lysis.

2.2.3. ARTEMESININ

ADMINISTRATION OF THE SESQUITERPENE LACTONE ARTEMISININ OR ITS ANALOGS. The therapeutic goal is to lyse tumor cells via generation of free radicals brought about by the interaction of artemisinin or its analogs with iron. [Prior PEFHT treatment may increase artemisinin saturation of

solid tumors by virtue of the fact that hyperthermia has been shown increase non-neurological vasodilation in cancerous tissue [16]. This effect, modulated by nitric oxide (NO) whose synthesis is catalyzed by Fe^{2+} released by ferritin, is dependent on the ferritin content of neolastic tissue. The iron-loading phase of this regimen (STEP NO. 2) helps assure that adequate tumor Fe^{2+} is available for NO synthesis. During hyperthermia therapy, cell-mediated immune responses are enhanced. These processes involve NO and subsequent free radical generation.

Note: Treatment efficacy may be enhanced by concomitant or follow-up use of certain chemotherapeutic drugs and immunopotentiating and/or immunoaugmentative agents.

BACKGROUND ON ARTEMISININ. Artemisinin is obtained from Artesmisia annua L. (Wormwood) and, along with its derivatives such as artesunate have been employed to successfully treat all forms of malaria. Artesunate possesses an endoperoxide bridge that reacts with iron (and especially heme) to form singlet oxygen as well as free radicals. Following oral administration, artesunate is rapidly absorbed and reaches C_{max} within 45-90 minutes. It is metabolized in the liver by hydrolysis to dihydroartemisinin, and has a very low level of toxicity.

ARTEMISININ AS AN ONCOLYTIC AGENT. In 1996 the United States Patent Office issued a patent to Henry C. Lai, and Narendra P. Singh (University of Washington, Seattle, Washington, USA), for the use of compounds having an endoperoxide moiety that is reactive with heme under conditions that enhance intracellular iron concentrations. Among the compounds listed in this patent are artemisinin, dihydroartemisinin, artemether, arteether, artesunate, and artesunate salts [17].

The BACKGROUND section includes a citation of studies (mostly in vitro) indicating that various artemisinin derivatives were cytotoxic to specific tumor cell lines such as Erlich ascites tumor cells, while artemisinin alone has been shown to be toxic to cancer cells in vitro at 20 to 180 μM range [18], and it was described that they were "more effective for hepatoma and embryonic lung cells than against human gastric cancer cells".

Several examples were furnished to indicate the oncolytic effects of artemisinin and dihydro-artemisinin, and two of them are briefly cited below:

(i) An in vivo study involving a 7-year-old canine (basset hound) with lymphosarcoma of the lymph nodes. The animal was treated with artemisinin (10 mg/day) and ferrous sulfate (10 mg/day p.o.) according to a specific schedule. Lymph nodes in multiple sites shrunk, many significantly so. When the treatment was stopped, all nodes increased in size. The animal survived 5 months post-treatment, then was destroyed [19].

(ii) An in vivo study was carried out on a female canine retriever that had been operated on for hemangiopericytoma of the right thigh. Treatment with artemisinin (10 mg/day) and ferrous sulfate (10 mg/day p.o.) was begun on the animal one week following surgery, and was continued for 23 consecutive days. No tumor recurrence was observed during or at 3 months following treatment cessation [20].

It is also of note that Lai and Singh reported in 2001 a pronounced oncolytic effect in breast cancer cells treated with holotransferrin and dihydroar-temisinin.

2.2.4. DECREASING IGF-I

IGF-I has been demonstrated to modulate cell proliferation, apoptosis and tumorigensis [21]. Many tumors have cell surface receptors for IGF-I which makes them especially likely to respond to any intervention aimed at reducing IGF-I levels or blocking IGF-I receptors. Interestingly, dietary restriction appears to lower IGF-I and thus impact cancer progression. In experiments involving heterozygous p53 mice, the animals were given p-cresidine to induce preneoplasia. Following confirmation of the existence of bladder urothelial preneoplasia in the mice, they were divided into 3 treatment groups: one group with no dietary restrictions (DR), one group with a 20% dietary restriction; and one goup with a 20% dietary restriction plus IGF-I. Tumor progression was decreased by dietary restriction, and subsequent restoration of IGF-I levels increased the stage of the tumors. Furthermore, apoptosis in the preneoplastic lesions was ten times higher in the mice who

underwent dietary restriction compared to the other two groups [22].

Likewise, several studies have shown that antagonists of growth hormone-releasing hormone (GH-RH) such as the experimental drug JV-1-38 inhibit various neoplasia indirectly through interference with the GH-IGF I axis and directly through the suppression pf autocrine/paracrine IGF-I, IGF-II, or GH-RH [23].

Until such time as GH-RH antagonists are FDA approved, there are naturally occurring polyphenols in soy and tomatoes that have been shown to reduce IGF-I and are available as dietary supplements. Among these are genistein, quercitin and daidzein. The diet section purposely includes foods that contain these compounds. In addition, pure genistein should be employed liberally, as it exhibited dose-dependent modulation of IGF-I with resultant inhibition of tumor growth in at least one in vitro experiment (50% inhibitory concentration of 25-40 μM in the AT6.3 rat prostate cancer cells). It also potently induced G_2/M cell cycle arrest in these cancer cells [24].

3. CONCLUDING REMARKS

The regimen outlined in this paper exploits specific, crucial pathways in the life-sustaining biology of many tumor cell types in such a way as to afford a window of opportunity for effecting oncolysis. Notable animal and human use of methionine restriction to treat tumors plus case history successes reported using intracellular pathway-compromising metabolic oncolytic approaches (The Cone Method, The Metabolic Oncolytic Regimen) suggest that the MSTOR should make tumor shrinkage and perhaps total lysis more probable. The fact it can be employed as a complement to standard therapies or as a "stand alone" measure for end-stage terminal patients underscores its potential utility.

REFERENCES

[1] PAYNE AG [2001] The Revised Metabolic Oncolytic Regimen for Effecting Lysis in Solid Tumors. Townsend Letter for Doctors 217: 78-85.

[2] SCHNELLDORFER T, GANSAUGE S, GANSAUGE F, SCHLOSSER S, BEGER HG AND NUSSLER AK [2000] Glutathione depletion causes cell growth inhibition and enhanced apoptosis in pancreatic cancer cells. Cancer 89: 1440-1447.

[3] EPNER DE, MORROW S, WILCOX M AND HOUGH JL [2002] Nutrient intake and nutritional indexes in adults with metastatic cancer on a phase I clinical trial of dietary methionine restriction. Nutr. Cancer 42: 158-166.

[4] SUGIYAMA T, SADZUKA Y, TANAKA K AND SONOBE T [2001] Inhibition of glutamate transporter by theanine enhances the therapeutic efficacy of doxorubicin. Toxicol Lett 121: 89-96.

[5] SADZUKA Y, SUGIYAMA T, SUZUKI T AND SONOBE T [2001] Enhancement of the activity of doxorubicin by inhibition of glutamate transporter. Toxicol Lett 123: 159-167.

[6] FUJIWARA K AND WATANABE T [1990] Effects of hyperthermia, radiotherapy and thermoradiotherapy on tumor microvascular permeability. Acta Pathol Jpn 40: 79-84.

[7] BABINCOVA M, LESZCZYNSKA D, SOURIVONG P AND BABINEC P [2000] Selective treatment of neoplastic cells using ferritin-mediated electromagnetic hyperthermia. Med Hypoth 54: 177-179.

[8] MITSUMORI M, HIRAOKA M, SHIBATA T, OKUNO Y, NAGATA Y, NISHIMURA Y, ABE M, HASEGAWA M, NAGAE H AND EBISAWA Y [1996] Targeted hyperthermia using dextran magnetite complex: a new treatment modality for liver tumors. Hepatogastroenterology 43: 1431-1437.

[9] YANASE M, SHINKAI M, HONDA H, WAKABAYASHI T, YOSHIDA J AND KOBAYASHI T [1997] Intracellular hyperthermia for cancer using magnetite cationic liposomes: ex vivo study. Jpn J Cancer 88: 630-632.

[10] COOK JD AND REDDY MB [2001] Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. Am J Clin Nutr 73: 93-98.

[11] HALLBERG L, BRUNE M AND ROSSANDER-HULTHEN L [1987] Is there a physiological role of vitamin C in iron absorption? Ann N Y Acad Sci 498: 324-332.

[12] MOUTAFCHIEV DA AND SIRAKOV LM [1992] Effect of insulin and adrenaline on the ^{59}Fe transferrin uptake of lactating mouse mammary gland cells. Horm Metab Res 24: 420-423.

[13] DAVIS RJ, CORVERA S AND CZECH MP [1986] Insulin stimulates cellular iron uptake and causes the redistribution of intracellular transferrin receptors to the plasma membrane. J Biol Chem 261: 8708-8711.

[14] MONTEIRO, HP, WINTERBOURNE CC AND STERN A [1991] Tetravalent vanadium releases ferritin iron whivh stimulates vanadium-dependent lipid peroxidation. Free Radic Res Commun 1:125-129.

[15] ibid.

[16] ANBAR M [1994] Hyperthermia of the cancerous breast: analysis of mechanism. Cancer Lett 84: 23-29.

[17] LAI HC AND SINGH NP [1996] Methods of inhibition or killing cancer cells using an endoperoxide. United States Patent No. 5,578,637.

[18] ibid.

[19] ibid.

[20] ibid.

[21] DUNN SE, KARI FW, FRENCH J, LENINGER JR, TRAVLOS G, WILSON R AND BARRETT JC [1997] Dietary restriction reduces insulin-like growth factor I levels, which modulates apoptosis, cell proliferation, and tumor progression in p-53 deficient mice. Cancer Res 57: 4667-4672.

[22] ibid.

[23] CSERNU VJ, SCHALL AV, KIARI H AND ARMATI P [1999] Inhibition of growth, production of insulin-like growth factor-II (IGF-II), and expression of IGF-II mRNA of human cancer cell lines by antagonistic analogs of growth hormone-releasing hormone in vitro. Proc Natl Acad Sci USA 96: 3098-3103.

[24] WANG S, DEGROFF VL AND CLINTON SK [2003] Tomato and soy polyphenols reduce insulin-like growth factor-I-stimulated rat prostate cancer cell proliferation and apoptotitic resistance in vitro via inhibition of extracellular signaling pathways involving tyrosine kinase. J Nutr 133: 2367-2376.

RECEIVED 6-4-2004.

ACCEPTED 8-24-2004.