Autophagy 10:8, 1478–1480; August 2014; © 2014 Landes Bioscience

Outcome of early clinical trials of the combination of hydroxychloroquine with chemotherapy in cancer

Andrew Poklepovic[1] and David A Gewirtz[2,*]

[1]Division of Hematology and Oncology; Virginia Commonwealth University; Massey Cancer Center; Richmond, VA USA; [2]Department of Pharmacology, Toxicology and Medicine; Virginia Commonwealth University; Massey Cancer Center; Richmond, VA USA

The premise of inhibiting autophagy to overcome resistance to chemotherapy has been investigated in 5 clinical phase I trials combining hydroxychloroquine with vorinostat, temsirolimus, temozolomide, or bortezomib. These studies have provided a number of insights relating to the tolerability of the combination treatments. In addition, these studies should provide guidance in the planning and design of future trials to directly determine whether the strategy of autophagy inhibition could prove useful in the treatment of various malignancies.

Keywords: autophagy, hydroxychloroquine, temozolomide, temsirolimus, vorinostat, bortezomib

Abbreviations: CTCs, circulating tumor cells; GBM, glioblastoma; HCQ, hydroxychloroquine; PBMCs, peripheral blood mononuclear cells

Submitted: 05/23/2014

Revised: 05/29/2014

Accepted: 06/02/2014

Published Online: 06/12/2014

http://dx.doi.org/10.4161/auto.29428

*Correspondence to David A Gewirtz; Email: gewirtz@vcu.edu

The premise of inhibiting autophagy to overcome chemotherapeutic resistance is based on extensive preclinical data both in tumor cells in culture and in tumor-bearing animal models that have suggested that autophagy induced by antitumor drugs and radiation is cytoprotective in function and can be exploited for therapeutic benefit. The role of autophagy in cancer has been extensively studied for the past 15 y, and the transition to clinical research with modulators of autophagy is now actively underway. The 5 studies of hydroxychloroquine (HCQ) presented in this issue of *Autophagy* represent some of the first clinical data from studies combining autophagy inhibitors with chemotherapy, and begin to answer some preliminary questions regarding the role of autophagy in cancer therapeutics, although many issues remain unresolved.

Since autophagy can act in both a protective or a toxic fashion depending on the stimulus and the cellular target, and in some cases may have neither a cytoprotective nor cytotoxic function,[1] the concurrent development of reliable biomarkers of autophagy assumes great importance. It is suggested from the study of vorinostat with HCQ by Mahalingam et al.[2] that analysis of peripheral blood mononuclear cells (PBMCs) may not reflect the degree of autophagy inhibition in tumors. Although the number of tumor biopsies in this study was small, there was a stark contrast in the degree of autophagy observed within the tumor specimens compared with what was seen in PBMC analysis. While markers of autophagy were identified in PBMCs in the highest dose cohort in this study, that dose proved to be intolerable to patients.

The studies of temsirolimus with HCQ by Rangawala et al.[3] and of dose intense temozolomide with HCQ also by Rangawala et al.[4] were able to achieve higher doses of HCQ administration, and increased autophagic vacuole formation was observed in PBMCs at the highest dose levels in combination with the chemotherapy treatments. The degree of autophagy inhibition in the tumor, however, was not determined at lower dose levels when autophagy inhibition was not identified in PBMCs. It remains unknown if these tumors also experience autophagy inhibition at doses of HCQ that are too low to induce autophagic vacoule formation in PBMCs. Furthermore, temsirolimus alone did not demonstrate autophagy induction in the PBMCs or the tumor cells (although temsirolimus alone was only shown at 4 h in the tumors).

In the studies by Rosenfeld et al.[5] an increase an autophagic vacuoles in PBMCs is shown over the course of 9 wk treatment with temozolomide, radiation, and hydroxychloroquine; however, the increase did not achieve significance and the capacity of either temozolomide or radiation alone to promote autophagy was not evaluated. In contrast, in the studies by Vogl et al.,[6] increased vacoule formation is detected in bone marrow plasma cells for hydroxychloroquine alone and hydroxychloroquine + bortezomib but not in PBMCs. Taken together, these reports clearly suggest that surrogate measurements of autophagy are not likely to be highly informative. Furthermore, it cannot be determined whether the chemotherapeutic drug alone promotes autophagy, which should ostensibly be one of the fundamental criteria for attempting to improve the therapeutic response through autophagy inhibition.

At this stage of clinical development, validation of the role of HCQ for inhibiting autophagy in tumors is paramount, and sampling of tumors accessible to serial biopsies is very important. As techniques in circulating tumor cell analysis continue to advance, it may be important to compare autophagy in circulating tumor cells (CTCs) to tumor biopsies, as CTC analysis offers investigators easier access to tumor cells undergoing metastatic dissemination. However, as is the case with PBMCs and primary tumor specimens, the autophagy in CTCs may not correlate with autophagy in the bulk tumor microenvironment, and this may have significant clinical implications.

In marrow-derived cancers, the study by Vogl et al.[6] evaluating bortezomib with HCQ did demonstrate that the bone marrow compartment and autophagic response to HCQ are similar to what was seen in visceral tumor analysis. Fortunately, it does appear that there are clear and consistent methods across studies to evaluate the presence of autophagy with electron microscopy. However, as indicated by Rangwala et al.,[4] there is the larger issue of what an increase of autophagic vacuoles in tumors actually means in relation to autophagy induction and/or inhibition. In this study, the combination of temozolomide and HCQ showed accumulations of autophagic vacuoles following 6 wk of therapy. Whether this was due to effective inhibition of the completion of autophagy by HCQ, induction of autophagy by temozolomide, or a combination of both, was unclear. Randomized trials with adequate correlative tumor tissue analysis would be required to answer this question.

As might have been anticipated in the case of early trials, none of these studies was designed to directly address the question of whether inhibition of autophagy in cancer can overcome chemotherapeutic resistance. All of these studies, except for the phase I/II study of temozolomide with HCQ in glioblastoma (GBM) by Rosenfeld et al.,[5] were dose finding in nature. In this GBM study, it was apparent that there was marked, early, and profound bone marrow suppression occurring with continuous temozolomide with HCQ administered at 800 mg. This is in contrast with the phase I study by Rangwala et al.,[4] wherein higher doses of both temozolomide and HQC were tolerated, albeit in an intermittent schedule. The cumulative dose of temozolomide in both the Rosenfeld et al.[5] and Rangwala et al.[4] studies is the same, with the major differences being intermittent dosing in Rangwala's study, and concurrent radiotherapy in Rosenfeld's study. It is not yet known what effect autophagy inhibition will have on the toxicity of radiotherapy; this question may be answered in part by ongoing clinical trials evaluating HCQ and radiotherapy (NCT01417403, NCT01602588, NCT01494155), although none will address the effects of long-course radiotherapy with the addition of HCQ alone. It seems less likely that central nervous system radiotherapy potentiated bone marrow toxicity from the drug combination, as the skull is not a major source of hematopoiesis. The profound bone marrow toxicity seen in the study by Rosenfeld et al.[5] suggests that the autophagy inhibition with continuous therapy may lead to dangerous hematological toxicity at higher doses, while intermittent therapy may allow for dose intensification. This hypothesis is supported by the finding of a similarly lower maximum tolerated dose of HCQ in the Mahalingam et al. study[2] of continuous vorinostat with HCQ. The intermittent dosing of bortezomib in the Vogl et al. study,[6] and the intermittent dosing of temsirolimus in the Rangwala et al. study[5] allowed for dose escalation similar to the dose intense temozolomide study by Rangwala et al.[5] This may be an effect of the chemotherapy instead of the HCQ, given that the half-life of HCQ is long and achievement of steady-state levels takes weeks, not days. When reviewing the toxicity data from the studies, the potentiation of chemotherapy toxicity did not seem to be present to the same degree regarding nonhematological toxicity. HCQ nonhematological toxicity, however, at least at the lower doses used by rheumatologists (200 mg twice a day by mouth), generally manifests over a period of years, not months.[7,8] It may be that with more prolonged dosing there will be increased nonhematological toxicity, and late toxicities including retinopathy and myopathy, among others, should be considered as potential late toxicity events.[7,8] The nature of the hematological toxicity may require HCQ dose modifications in other clinical trials as well.

This raises a question, which may be able to be answered in preclinical models and explored in clinical trials with robust biomarker assays. Does intermittent intense inhibition of autophagy with HCQ modulate chemotherapeutic effectiveness differently than continuous, less-intense inhibition? A potential clinical trial would be to evaluate 2 dose schedules of temozolomide with HQC, with a control arm: Cohort A 75 mg/m^2 with 600 mg by mouth daily continuously, cohort B 150 mg/m^2 d 1–7, with HCQ at 600 mg twice a d by mouth. Pre- and post-biopsies would be required in both groups. This type of study could be performed in sarcoma or melanoma, as temozolomide has activity in both tumor types, and would likely have disease amenable to biopsy. A third arm serving as a control arm would be most appropriate to gauge the baseline effect on autophagy from temozolomide, and may begin to answer some of the questions raised by Rangwala et al.[4] in the study of dose-intense temozolomide with HCQ. This type of trial would also allow for a comparison of efficacy.

In the Rosenfeld study in GBM[5] it is unclear if the drug effect was too small

to enhance the antitumor activity of temozolomide, or if the strategy is not a valid one in this disease. All we are able to conclude is that adding HCQ at 600 mg/d to continuous temozolomide with radiotherapy is insufficient to improve survival. Similar doubts about chemotherapeutic effectiveness were identified in the Vogl et al. myeloma study[6] where the only patients with robust responses were 3 patients who were bortezomib naive. Single agent bortezomib has over a 40% complete response + partial response rate in untreated patients. It may be that in multiple myeloma, the best way to explore augmentation of chemotherapeutic effectiveness would be a phase I trial of a standard regimen such as VRD (bortezomib + lenalidomide + dexamethasone), with addition of HCQ. A phase I study would be required, because lenalidomide is dosed continuously for 3 wk every 28 d, and the continuous dosing may lead to more pronounced toxicity as seen in other studies. Once that dose is known, a comparative study with response as the endpoint may be able to answer the question.

The discussion section of the report by Mahalingam et al.[2] et al. makes a number of critical points that are worth reemphasizing. One is that there is uncertainty as to whether HCQ actually inhibits autophagy in human tumors (at doses that are tolerable) and whether the extent of inhibition would be sufficient to alter chemosensitivity. As indicated above, the detection of autophagic vacuole accumulation in a tumor cell frequently does not actually indicate whether autophagy is being induced or inhibited. Another is the need to identify patients who are likely to benefit from therapy with autophagy inhibitors; this will unquestionably require the development of clinical biomarkers that would indicate whether the treatment of a particular malignancy with a selected drug (or radiation) is actually promoting the cytoprotective form of autophagy that likely would be susceptible to modulation by autophagy inhibition.

It should be recognized that it is very early in the clinical development of inhibitors of autophagy and that autophagy remains incompletely understood as a mechanism of tumor cell survival in human patients. Toxicity may be modulated based upon the schedule and choice of concurrent agents. Additional mechanistic studies in preclinical models are clearly required. In addition, it might be prudent to develop a consensus based on preclinical data as to which types of cancer and which class or classes of drugs used in standard regimens might be most appropriate for testing in the context of clinical trials of HCQ or other modulators of autophagy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Gewirtz DA. The four faces of autophagy: implications for cancer therapy. Cancer Res 2014; 74:647-51; PMID:24459182; http://dx.doi.org/10.1158/0008-5472.CAN-13-2966
2. Mahalingam D, Mita M, Sarantopoulos J, Wood L, Amaravadi R, Davis L, Mita A, Curiel TJ, Espitia CM, Nawrocki ST, et al. Combined autophagy and HDAC inhibition: A phase I safety, tolerability, pharmacokinetic, and pharmacodynamic analysis of hydroxychloroquine in combination with the HDAC inhibitor vorinostat in patients with advanced solid tumors. Autophagy 2014; 10:1403-14; PMID:24991835; http://dx.doi.org/10.4161/auto.29231.
3. Rangwala R, Chang C, Hu J, Algazy K, Evans T, Fecher L, Schuchter L, Torigian DA, Panossian J, Troxel T. Combined MTOR and autophagy inhibition: Phase I trial of hydroxychloroquine and temsirolimus in patients with advanced solid tumors and melanoma. Autophagy 2014; 10:1391-1402; PMID:24991838; http://dx.doi.org/10.4161/auto.29119
4. Rangwala R, Leone R, Chang C, Fecher L, Schuchter LM, Kramer A, Tan K-S, Hetjian DF, Rodgers G, Gallagher M, et al. Phase I trial of hydroxychloroquine with dose-intense temozolomide in patients with advanced solid tumors and melanoma. Autophagy 2014; 10:1369-79; PMID:24991839; http://dx.doi.org/10.4161/auto.29118
5. Rosenfeld MR, Ye X, Supko JG, Desideri S, Grossman SA, Brem S, Mikkelson T, Wang D, Chang C, Hu J, et al. A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. Autophagy 2014; 10:1359-68; PMID:24991840; http://dx.doi.org/10.4161/auto.28984
6. Vogl DT, Stadtmauer EA, Tan KS, Heitjan DF, Davis LE, Pontiggia L, Rangwala R, Piao S, Chang YC, Scott EC, et al. Combined autophagy and proteasome inhibition: A phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma. Autophagy 2014; 10:1380-90; PMID:24991834; http://dx.doi.org/10.4161/auto.29264
7. Kwon JB, Kleiner A, Ishida K, Godown J, Ciafaloni E, Looney RJ Jr. Hydroxychloroquine-induced myopathy. J Clin Rheumatol 2010; 16:28-31; PMID:20051753; http://dx.doi.org/10.1097/RHU.0b013e3181c47ec8
8. Stelton CR, Connors DB, Walia SS, Walia HS. Hydrochloroquine retinopathy: characteristic presentation with review of screening. Clin Rheumatol 2013; 32:895-8; PMID:23515601; http://dx.doi.org/10.1007/s10067-013-2226-2