Fatal toxicity of chloroquine or hydroxychloroquine with metformin in mice

N.V Rajeshkumar[1], Shinichi Yabuuchi[2], Shweta G. Pai[2], Anirban Maitra[3], Manuel Hidalgo[4] and Chi V. Dang[5]

[1]Department of Oncology, Pathology, Radiation Oncology and Molecular Radiation Sciences, Johns Hopkins University School of Medicine, Baltimore, MD.

[2]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD.

[3]Sheikh Ahmed Center for Pancreatic Cancer Research, M.D. Anderson Cancer Center, Houston, TX.

[4]Division of Hematology and Medical Oncology, Weill Department of Medicine, Sandra and Edward Meyer Cancer Center, Weill Cornell Medicine, New York, NY.

[5]Ludwig Institute for Cancer Research, New York, NY.

Corresponding authors: NV Rajeshkumar, Ph.D, Department of Radiation Oncology and Molecular Radiation Sciences, Johns Hopkins University School of Medicine, 1550 Orleans Street, CRB II, Room 443, Baltimore, MD 21287. Phone: 410-955-7312; Fax: 410-502-2821 E-mail: rnv1@jhmi.edu

Chi V. Dang, MD. Ph.D, Ludwig Institute for Cancer Research, 666 Third Avenue, 28th Floor, New York, NY 10017. Phone: 212-450-1566; Fax: 212- 450-1545; E-mail: cdang@licr.org

Abstract

Guided by the principle of *primum non nocere* (first do no harm), we report a cautionary note on the potential fatal toxicity of chloroquine (CQ) or hydroxychloroquine (HCQ) in combination with anti-diabetic drug metformin. We observed that the combination of CQ or HCQ and metformin, which were used in our studies as potential anti-cancer drugs, killed 30-40% of mice. While our observations in mice may not translate to toxicity in humans, the reports that CQ or HCQ has anti-COVID-19 activity, the use of CQ resulting in toxicity and at least one death, and the recent Emergency Use Authorization (EUA) for CQ and HCQ by the US Food and Drug Administration (FDA) prompted our report. Here we report the lethality of CQ or HCQ in combination with metformin as a warning of its potential serious clinical toxicity. We hope that our report will be helpful to stimulate pharmacovigilance and monitoring of adverse drug reactions with the use of CQ or HCQ, particularly in combination with metformin.

Introduction. Guided by the principle of *primum non nocere* (first do no harm), we report a cautionary note on the potential fatal toxicity of chloroquine (CQ) or hydroxychloroquine (HCQ) in combination with anti-diabetic drug metformin. We observed that the combination of CQ or HCQ and metformin, which were used in our studies as potential anti-cancer drugs, killed 30-40% of mice. While our observations in mice may not translate to toxicity in humans, the reports that CQ or HCQ has anti-COVID-19 activity [1], the use of CQ resulting in toxicity and at least one death, and the recent Emergency Use Authorization (EUA) for CQ and HCQ by the US Food and Drug Administration (FDA) prompted our report. Here we report the lethality of CQ or HCQ in combination with metformin as a warning of its potential serious clinical toxicity. We hope that our report will be helpful to stimulate pharmacovigilance and monitoring of adverse drug reactions with the use of CQ or HCQ, particularly with metformin.

Methods. Animal experiments were conducted following approval by the Animal Care and Use Committee guidelines of the Johns Hopkins University (Baltimore, MD). Tumor bearing or non-tumor bearing immunocompromised mice were injected with 100 μL of saline vehicle, chloroquine (CQ, 60 mg/kg), hydroxychloroquine (HCQ, 60 mg/kg) and/or metformin (250 mg/kg) once daily intraperitoneally daily for 4 weeks as described [2 3]. A combination of CQ and metformin in the above-mentioned dose and frequency was administered to animals in the combination treatment group. In a separate study, non-tumor bearing immunocompromised and immunocompetent mice were treated with HCQ and metformin once daily in the above-mentioned dose for 38 days. Blood was drawn for chemistry and hematology via cardiac puncture, and organs and tissues were harvested, examined and processed for transmission electron microscopy as described [3].

Results. Based on our previous findings that metformin and CQ or HCQ curbed the growth of human pancreatic xenografts in athymic nude mice [2 3], we sought to determine whether the combination of metformin, which inhibits mitochondrial Complex I, and CQ that inhibits autophagy could be synergistic as an anti-cancer metabolic cocktail. In contrast to single agent

metformin or CQ, which have anti-tumor activity, we found that the combination of metformin and CQ was lethal in 40% of tumor bearing or non-tumor bearing mice (**Figure 1A**).

To determine whether HCQ is similarly lethal in combination with metformin, we tested the metformin+HCQ combination in non-tumor bearing nude mice, which showed a 40% mortality (**Figure 1B**). To determine whether immunocompromised nude mice were particularly sensitive to the combination, we treated immunocompetent C57BL/6 mice with the metformin+HCQ, which resulted in 30% lethality.

We then sought to determine the basis for the toxicity of metformin+HCQ combination in autopsy studies and found that body weights were not significantly different (**Table 1**) among the different groups. While organ weights were not different among the groups, we observed via transmission electron microscopy an increase in the number of autophagosomes in the heart, liver and kidneys of athymic nude mice treated with metformin+HCQ combination (**Figure 1C**). While the hematological findings were not different among the groups, we found that lactate dehydrogenase (LDH) and creatine kinase (CK) levels were elevated in all treatment groups as compared to control vehicle treated group (**Table 1**).

Discussion. Between March 16 and 21, 2020, partly based on a non-randomized study using HCQ with azithromycin [4] , claims disseminated through Twitter and amplified by the media that CQ or HCQ could be a therapy for COVID-19. Many individuals have started to take this drug, resulting in chloroquine poisoning in Nigeria (@NCDCgov #COVID19Nigeria) and a death in Arizona. Notably, CQ and HCQ doses which are used for the treatment of rheumatic diseases, could lead to the development of hypoglycemia, cardiomyopathy and retinopathy [5]. Here we report the lethality of metformin+CQ or +HCQ as a warning of its potential deadly toxicity, noting that the dosages in mice are similar to those in human with allometric scaling. Consistent with our findings, the combination of CQ and metformin resulted in CNS neuronal damage after cardiac arrest in rats [6]. We hope that our report will be helpful to stimulate

pharmacovigilance and monitoring of adverse drug reactions with the use of CQ or HCQ, particularly in combination with metformin.

Author contributions:

Concept and design: Rajeshkumar, Maitra, Hidalgo, Dang

Acquisition, analysis or interpretation of data: Rajeshkumar, Yabuuchi, Pai, Dang, Hidalgo

Drafting of manuscript: Dang, Hidalgo, Rajeshkumar

Statistical analysis: Rajeshkumar, Yabuuchi

Obtained funding: Dang, Maitra, Hidalgo, Rajeshkumar

Drs. Dang and Hidalgo have access to primary data in Dr. Rajeshkumar's possession.

Conflict of Interest Disclosures: MH, Stock and Ownership Interests: Champions Oncology, Pharmacyte Biotech, BioOncotech, Nelum, Agenus; Honoraria: Agenus, Erytech Pharma, Pharmacyte Biotech, Oncomatryx Biopharma S.L., IndMex, BioOncotech, Takeda; Consulting or Advisory Role: Oncomatryx Biopharma S.L., Takeda, Pharmacyte, Agenus, InxMed, BioOncotech; Research Funding: BiolineRx, Erytech Pharma; Patents, Royalties, Other Intellectual Property: Myriad Genetics. CVD, Stock: Agios Pharma; Board of Director member: Rafael Pharm; Consultant or Advisory: Dracen Pharm, Polaris Pharm, Geneos Pharm, Barer Institute. AM, Royalties: Cosmos Wisdom Biotechnology; License: Thrive Earlier Detection.

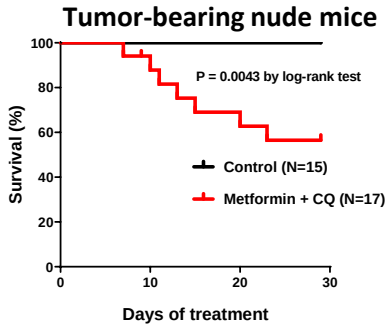
Funding/Support. This study was partially supported by funding from a Stand Up To Cancer Dream Team Translational Cancer Research grant (grant number: SU2C-AACR- DT0509; to N.V. Rajeshkumar, M. Hidalgo, A. Maitra and C.V. Dang). Dang is support by the Ludwig Institute for Cancer Research.

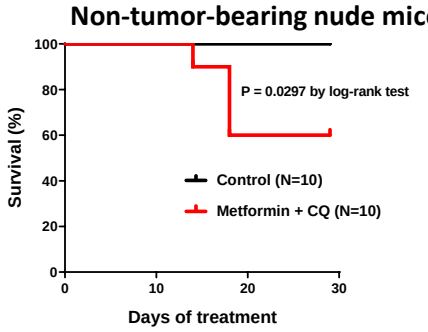
Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

REFERENCES

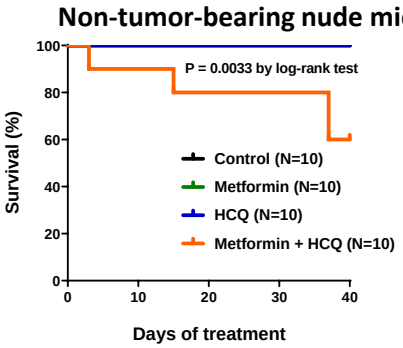
1. Cortegiani A, Ingoglia G, Ippolito M, Giarratano A, Einav S. A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. J Crit Care 2020 doi: 10.1016/j.jcrc.2020.03.005.
2. Rajeshkumar NV, Yabuuchi S, Pai SG, et al. Treatment of Pancreatic Cancer Patient-Derived Xenograft Panel with Metabolic Inhibitors Reveals Efficacy of Phenformin. Clin Cancer Res 2017;**23**(18):5639-47 doi: 10.1158/1078-0432.CCR-17-1115.
3. Yang A, Rajeshkumar NV, Wang X, et al. Autophagy is critical for pancreatic tumor growth and progression in tumors with p53 alterations. Cancer Discov 2014;**4**(8):905-13 doi: 10.1158/2159-8290.CD-14-0362.
4. Gautret P, Lagier JC, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020:105949 doi: 10.1016/j.ijantimicag.2020.105949.
5. Schrezenmeier E, Dorner T. Mechanisms of action of hydroxychloroquine and chloroquine: implications for rheumatology. Nat Rev Rheumatol 2020;**16**(3):155-66 doi: 10.1038/s41584-020-0372-x.
6. Zhu J, Liu K, Huang K, et al. Metformin Improves Neurologic Outcome Via AMP-Activated Protein Kinase-Mediated Autophagy Activation in a Rat Model of Cardiac Arrest and Resuscitation. J Am Heart Assoc 2018;**7**(12) doi: 10.1161/JAHA.117.008389.

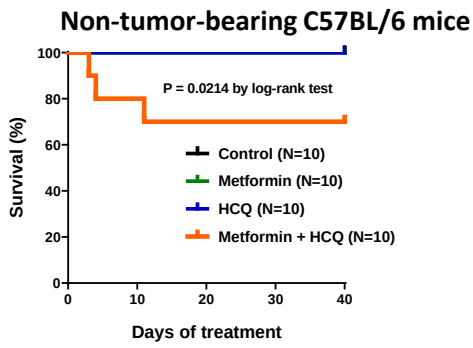
Figure 1 A


Tumor-bearing nude mice
Survival (%)
P = 0.0043 by log-rank test
Control (N=15)
Metformin + CQ (N=17)
Days of treatment

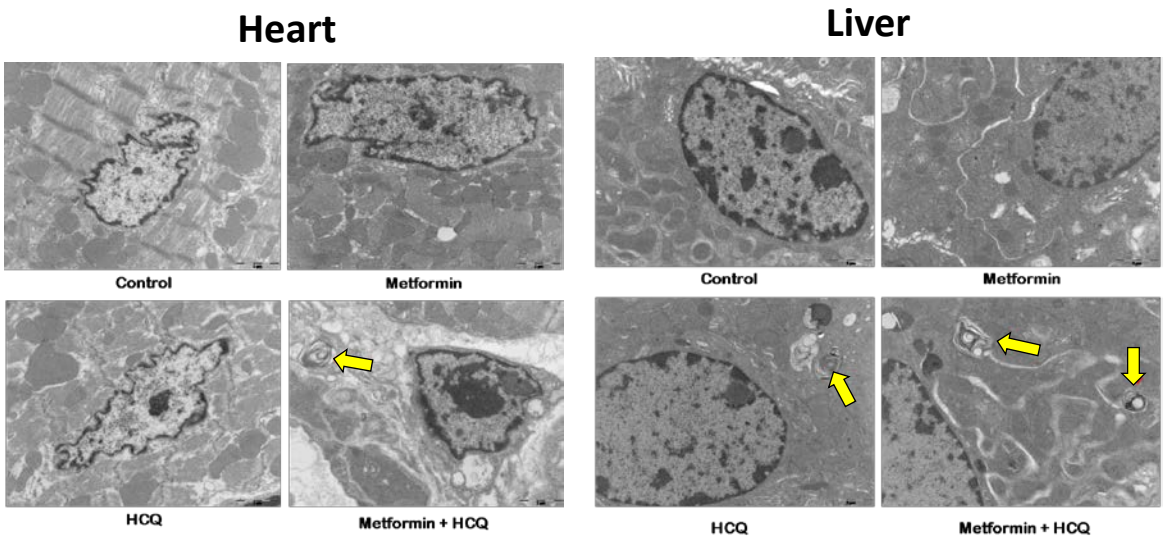

Non-tumor-bearing nude mice
Survival (%)
P = 0.0297 by log-rank test
Control (N=10)
Metformin + CQ (N=10)
Days of treatment

B


Non-tumor-bearing nude mice
Survival (%)
P = 0.0033 by log-rank test
Control (N=10)
Metformin (N=10)
HCQ (N=10)
Metformin + HCQ (N=10)
Days of treatment


Non-tumor-bearing C57BL/6 mice
Survival (%)
P = 0.0214 by log-rank test
Control (N=10)
Metformin (N=10)
HCQ (N=10)
Metformin + HCQ (N=10)
Days of treatment

C


Heart
Liver
Control
Metformin
HCQ
Metformin + HCQ
Control
Metformin
HCQ
Metformin + HCQ

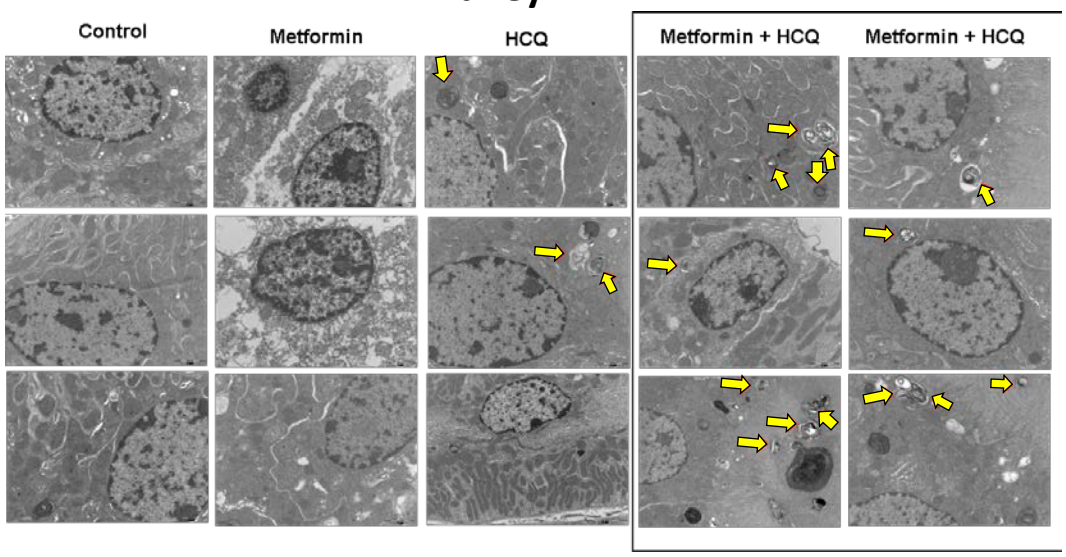

Kidney
Control
Metformin
HCQ
Metformin + HCQ
Metformin + HCQ

Table 1. Body weight, organ weight, CBC and clinical chemistry parameters of mice administered with Vehicle (Control), Metformin, HCQ or Metformin and HCQ.

Parameter	Unit	Non-tumor-bearing nude mice								Non-tumor-bearing C57B/L6 mice							
		Control		Metformin		HCQ		Metformin+HCQ		Control		Metformin		HCQ		Metformin+HCQ	
		Average	STDEV	Average	STDEV	Average	STDEV	Average	STDEV	Average	STDEV	Average	STDEV	Average	STDEV	Average	STDEV
Body weight	g	27.585	2.149	29.697	1.043	28.973	3.166	29.301	0.194	29.240	0.793	28.109	0.564	28.064	3.339	27.774	2.169
Organ weight																	
Liver	g	1.510	0.336	1.847	0.152	1.873	0.274	1.790	0.113	1.74	0.217	1.590	0.131	1.723	0.323	1.523	0.176
Spleen	g	0.123	0.023	0.143	0.021	0.153	0.042	0.117	0.006	0.067	0.012	0.107	0.064	0.097	0.015	0.070	0.010
Heart	g	0.220	0.035	0.223	0.038	0.167	0.040	0.173	0.006	0.173	0.015	0.183	0.006	0.167	0.032	0.187	0.012
R Kidney	g	0.297	0.051	0.360	0.026	0.360	0.020	0.303	0.032	0.293	0.015	0.297	0.012	0.290	0.046	0.277	0.029
L Kidney	g	0.307	0.023	0.353	0.031	0.343	0.046	0.307	0.031	0.250	0.01	0.263	0.031	0.260	0.035	0.257	0.015
CBC																	
WBC	K /uL	3.10	0.399	3.75	1.194	10.21	1.785	11.13	4.427	6.36	1.048	7.57	4.603	5.01	0.752	4.35	1.314
Neut	K /uL	1.43	0.483	1.19	0.433	4.37	0.709	3.57	0.548	1.77	1.039	2.88	3.590	1.29	0.168	1.04	0.477
Lymph	K /uL	1.57	0.137	2.27	0.585	3.68	1.325	6.28	3.907	4.15	0.497	3.95	0.196	3.52	0.633	3.00	0.775
Mono	K /uL	0.08	0.040	0.12	0.090	0.51	0.098	0.39	0.245	0.39	0.177	0.52	0.610	0.16	0.025	0.20	0.133
RBC	M /uL	9.81	0.419	9.42	0.104	9.02	0.525	8.83	0.285	9.09	0.199	8.95	0.915	9.18	0.184	10.11	0.492
Hb	g /dL	14.80	1.054	13.67	0.651	13.20	0.889	13.03	0.702	12.60	0.520	11.97	1.041	12.07	0.115	13.37	0.473
Hct	%	53.27	2.831	49.87	3.721	45.87	3.444	47.87	3.870	40.83	1.250	40.53	4.557	40.80	0.60	45.93	1.721
PLT	K /uL	1058.33	73.433	711.33	298.574	496.00	376.427	1094.67	188.988	1170.67	256.995	1206.67	86.524	1241.33	48.211	1183.33	120.101
Clinical Chemistry																	
Chol	mg /dL	140.33	13.577	173.00	2.646	150.00	4.359	131.33	10.693	128.00	3.00	114.33	5.033	112.33	9.074	120.33	7.234
Trig	mg /dL	108.00	17.578	103.67	13.503	109.00	30.790	169.33	90.473	97.33	5.508	113.67	30.746	97.33	18.610	93.00	31.241
UA	mg /dL	3.00	0.794	3.40	1.386	4.13	0.839	6.47	3.190	2.90	0.346	3.63	0.321	4.83	0.404	4.03	0.808
CK	U /L	206.33	78.806	**406.33**	**140.920**	**1084.00**	**454.175**	**452.67**	**164.214**	240	138.098	**215.00**	**102.504**	**503.67**	**349.632**	**205.67**	**97.828**
g-GTP	U /L	3.00	1.732	3.67	0.577	3.00	1.00	3.00	1.732	1.67	2.082	3.00	1.000	3.33	1.155	3.33	1.155
ALT	U /L	59.33	19.088	58.33	6.658	52.00	5.568	100.67	68.061	54.67	5.033	82.00	35.791	67.67	11.930	71.67	6.028
AST	U /L	103.33	21.385	163.00	17.349	219.67	4.163	233.67	122.786	92.67	23.159	187.33	79.387	168.00	32.419	145.33	9.504
Amyl	U /L	969.67	261.077	1205.00	122.135	1192.00	196.929	1313.33	129.732	1264.00	204.648	1607.33	383.981	1546.33	260.064	1563.00	78.848
ALP	U /L	112.00	11.000	121.67	10.599	96.33	11.015	74.67	8.737	75.33	3.055	90.67	31.974	84.33	3.512	87.67	16.862
T-Bil	mg /dL	0.20	0.000	0.23	0.058	0.27	0.058	0.30	0.100	0.43	0.058	0.27	0.058	0.23	0.058	0.23	0.058
Direct-Bil	mg /dL	0.10	0.000	0.17	0.058	0.27	0.115	0.20	0.100	0.47	0.058	0.27	0.058	0.17	0.058	0.20	0.100
Glu	mg /dL	227.67	44.970	225.67	31.943	257.00	40.951	356.33	117.619	237.67	42.028	192.67	75.936	244.00	23.065	261.00	64.257
T-protein	g /dL	5.20	0.173	5.47	0.231	5.40	0.173	4.50	0.781	6.00	0.10	5.63	0.153	5.87	0.058	5.63	0.252
Ca	mg /dL	10.73	0.153	10.77	0.643	11.17	0.306	11.73	1.686	10.10	0.436	10.17	0.451	10.63	0.231	10.20	0.100
BUN	mg /dL	20.67	1.528	22.33	3.055	19.00	5.292	17.33	3.786	19.00	1.732	15.67	4.726	14.00	3.606	13.33	1.528
Cre	mg /dL	0.40	0.000	0.37	0.058	0.33	0.058	0.33	0.058	0.20	0.2	0.30	0.000	0.43	0.058	0.40	0.000
Albumin	U /L	3.17	0.153	3.20	0.10	3.13	0.115	2.90	0.00	3.47	0.153	3.07	0.493	3.17	0.058	3.17	0.058
LDH	U /L	309.00	39.611	**3077.67**	**343.08**	**2665.67**	**319.639**	**2645.33**	**392.263**	478.33	54.372	**2884.00**	**1018.500**	**2879.67**	**533.209**	**2376.00**	**337.311**
HDL	mg /dL	82.67	8.622	97.33	3.51	84.33	4.041	80.33	3.786	78.33	3.055	65.00	10.440	67.00	6.245	68.67	1.528