Exploiting Innate Immune Cell Activation of a Copper-Dependent Antimicrobial Agent During Infection

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Inventory of Supplemental Information

Figure S1. Extracted Ion Chromatograms showing 8HQ is formed in activated RAW cells from QBP, related to Figure 1.

Figure S2. Calibration curves 8HQ in DMEM medium and RAW cells and % Recovery of 8HQ from cells, related to Figure 1.

Figure S3. 6HQ has no effect on growth of *C. neoformans*, related to Figure 2.

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Figure S5. 8HQ-copper complex does not change levels of iron or zinc, related to Figure 4.

Figure S6. Complete blood count of mice treated intraperitoneally with 8HQ and QBP for two weeks reveals no statistically significant effects on cell counts, related to Figure 6.

Extended Experimental Procedures

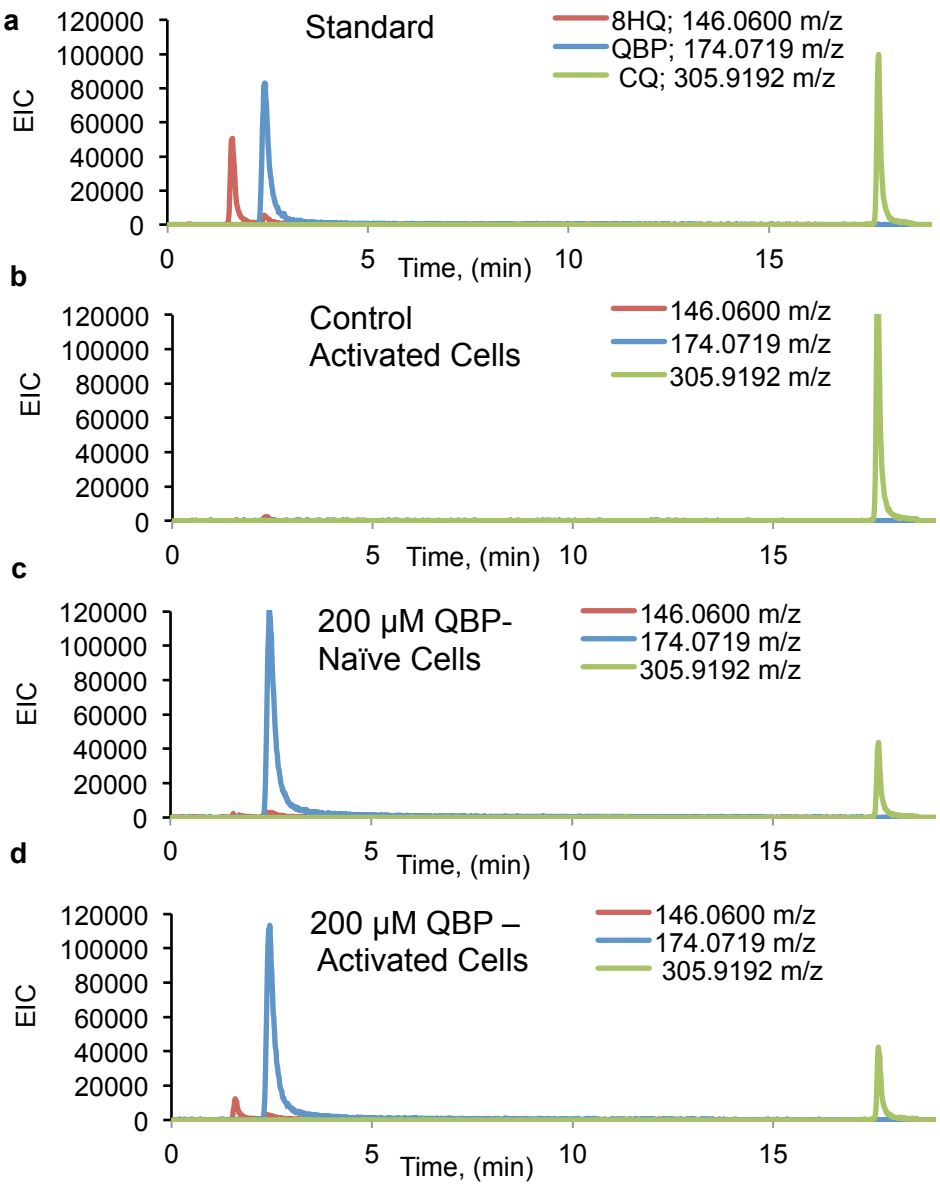
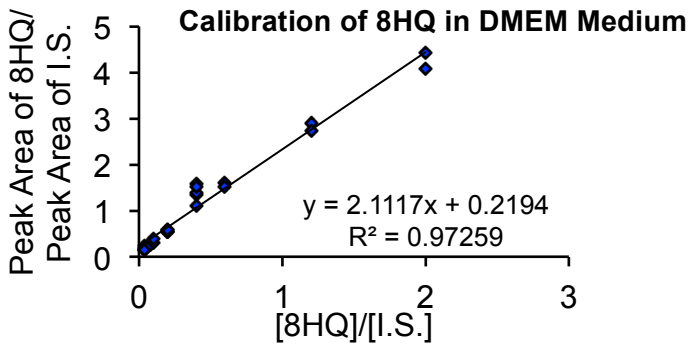


Figure S1 (related to Figure 1). Extracted Ion Chromatograms showing 8HQ is formed in activated RAW cells from QBP. (A) A standard of 8HQ, QBP and the internal standard, clioquinol of 5 µM, 20 µM and 25 µM, respectively in DMEM, 10% FBS, and penstrep was separated by LC-MS. The extracted-ion chromatogram (EIC) mode was used to identify and quantify 8HQ (protonated parent molecule $[M+H]^+$; m/z = 146.0600), QBP (analyzing in formic acid leads to pinanediol falling off to form quinoline-boronic acid, which was monitored $[M+H]^+$; m/z = 174.0719), and the internal standard, clioquinol ($[M+H]^+$; m/z =305.9192). (B) A control of activated RAW cells with no addition of QBP. 200 µM QBP was added to naïve (C) and activated (D) RAW 246.7 cells. 8HQ was only detected in activated RAW 264.7 cells as opposed to naive cells.

[8HQ]/[I.S.]	% Recovery	Standard Deviation
0.04	40.11	8.91
0.1	41.25	5.73
0.2	45.89	12.72
0.4	35.67	2.69
0.6	39.62	4.16

a



b

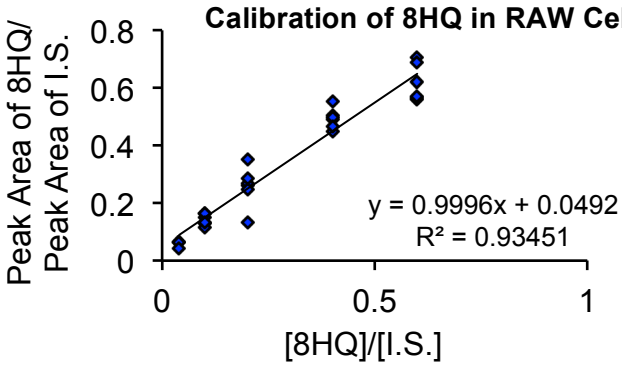


Figure S2 (related to Figure 1). Calibration curves 8HQ in DMEM medium and RAW cells and % Recovery of 8HQ from cells. For quantification purposes, calibration curves were made using 8-hydroxyquinoline (8HQ) as analyte and clioquinol as internal standard (I.S.) in either DMEM (A) or RAW cells (B) Quantification of 8HQ was performed by using analyte/internal standard peak area ratios. For calculating the conversion of 8HQ from QBP in RAW cells, quantification was performed by using the linear regression obtained from the calibration curve of 8HQ in RAW cells (C). The percent recovered from extracting 8HQ from RAW cells was determined by taking the ratio of peak areas of 8HQ/CQ in RAW cells over the peak areas of 8HQ/CQ DMEM.

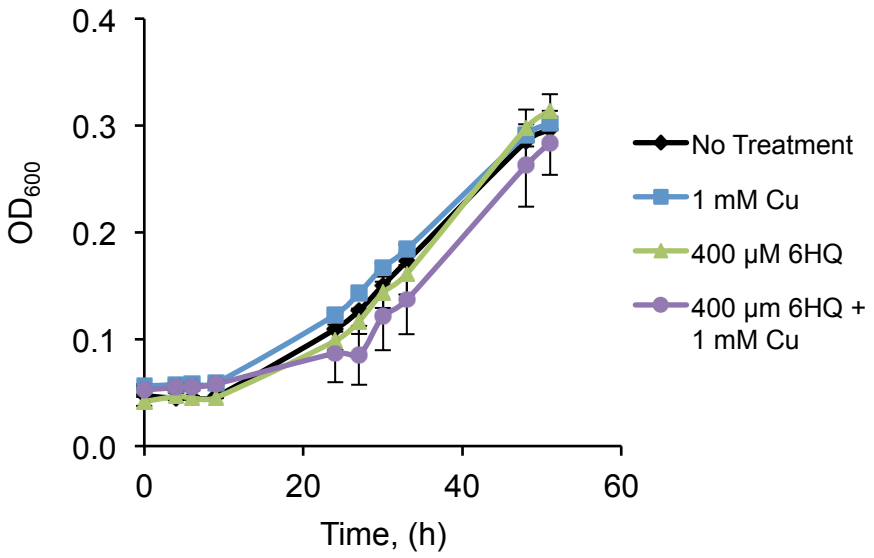


Figure S3 (related to Figure 2). 6HQ has no effect on growth of *C. neoformans*. Growth curves were performed with 400 µM 6HQ with or without 1 mM $CuSO_4$. The use of 6HQ where the hydroxyl group is moved from the 8-position to the 6-position on the quinoline leads to the elimination of toxicity even in the presence of 1 mM $CuSO_4$. The error bars represent the standard deviation (SD) of an experiment done in triplicate.

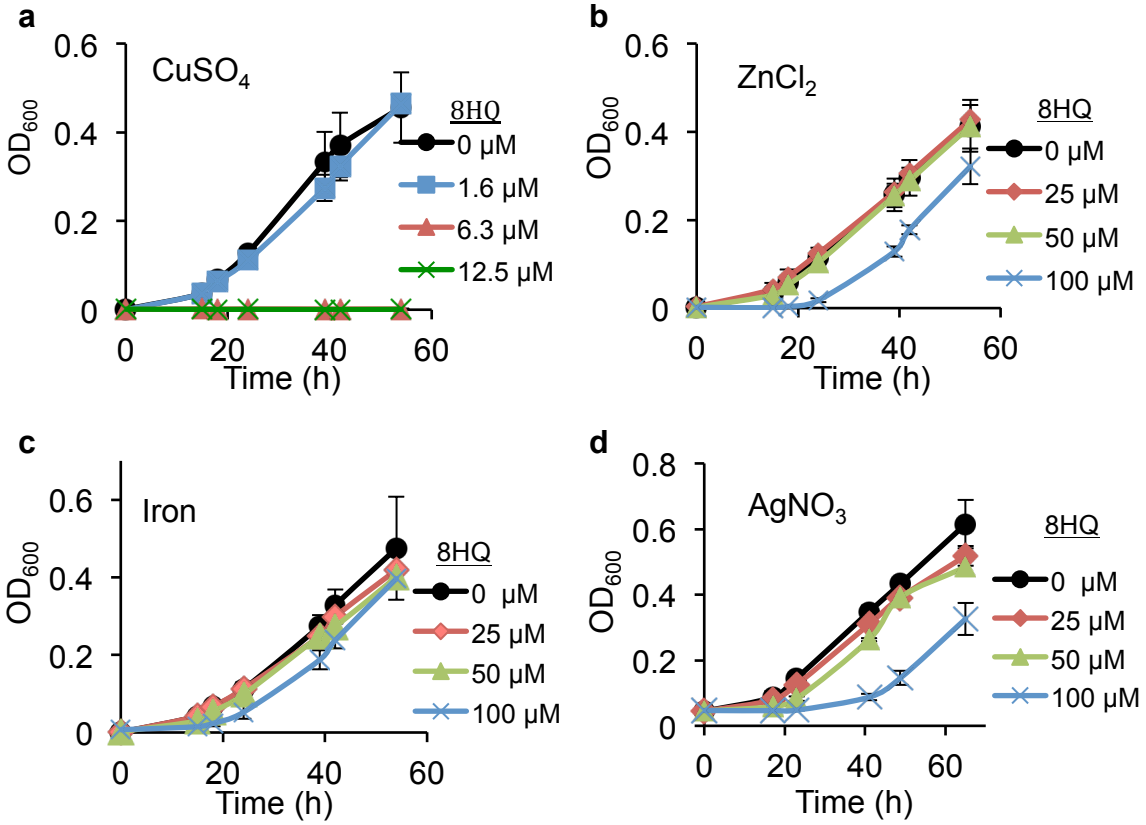


Figure S4 (related to Figure 2). An 8-HQ-copper complex is more toxic than complexes with other metals. Growth curves were performed in the presence of 8HQ and 10 μM (A) Cu, (B) zinc, (C) iron, (D) silver. Cu^{2+}, Zn^{2+}, Fe^{3+}, and Ag^{+} (provided as $CuSO_4$, $ZnCl_2$, ferric ammonium citrate, and $AgNO_3$). The error bars represent the SD of an experiment done in triplicate.

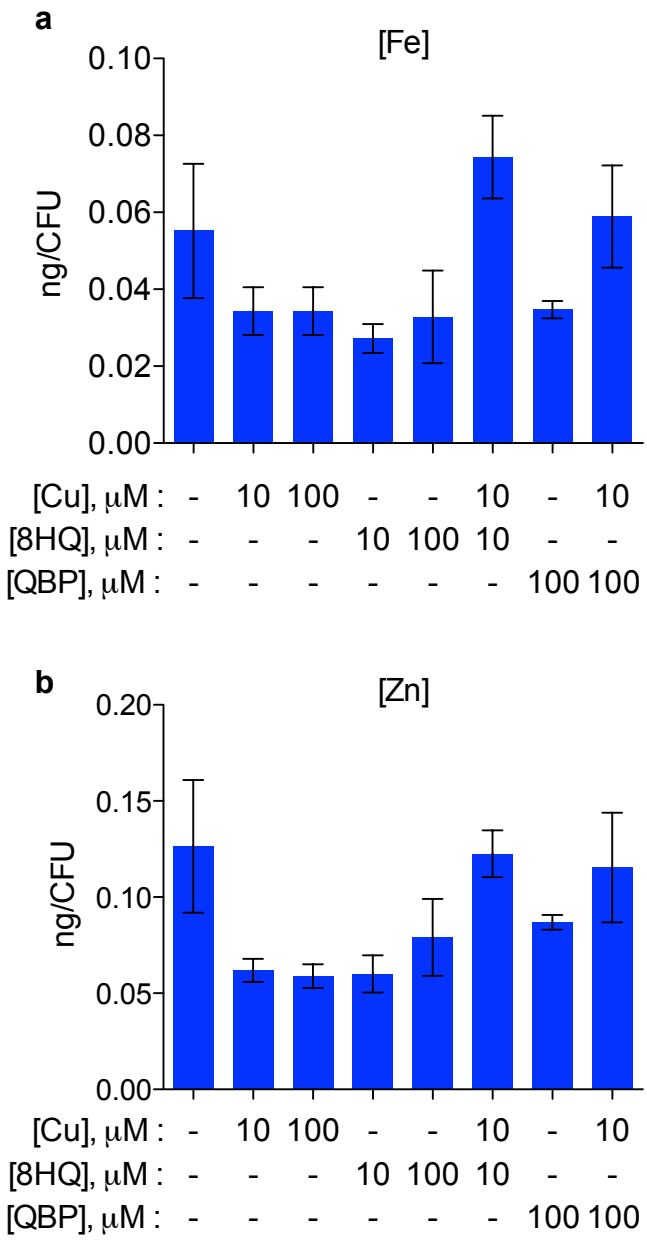


Figure S5 (related to Figure 4). 8HQ-copper complex does not change levels of iron or zinc. WT *C. neoformans* was treated with Cu and/or compound as indicated. After 1h, cells were harvested, digested and analyzed by ICP-MS. Concentrations of Fe and Zn were quantified as controls for Figure 4a, showing that the amounts of other metals were not affected by addition of Cu, 8HQ, or QBP. The error bars represent the standard error of the mean (SEM) from three experiments.

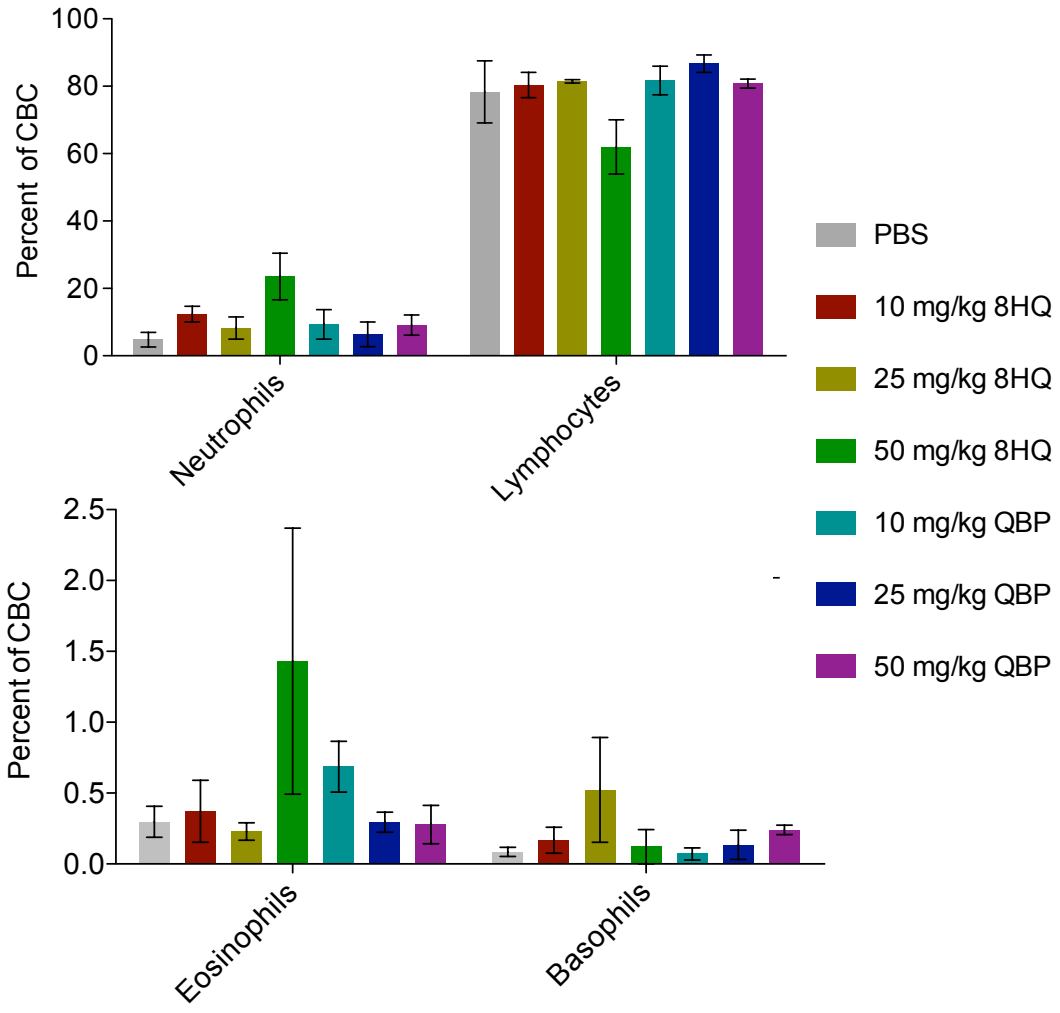


Figure S6 (related to Figure 6). Complete blood count of mice treated intraperitoneally with 8HQ and QBP for two weeks reveals no statistically significant effects on cell counts. A/J mice were intraperitoneally administered the indicated dose of 8HQ or QBP twice daily over the course of two weeks to test tolerable dose. After two weeks, blood samples were analyzed by the Blood Analysis Core Facility at Duke University. Shown are relative percent of each blood type in the conditions tested. While there was variation the changes were not statistically significant. The error bars represent the SEM of three mice per group.