รายงานวิจัยฉบับสมบูรณ์

โครงการการศึกษากลไกการออกฤทธิ์และกลไกการดื้อยา artemisinin

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สนับสนุนโดยสำนักงานคณะกรรมการอุดมศึกษา สำนักงานกองทุนสนับสนุนการวิจัย
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Abstract

Project Code : MRG5580085

Project Title : Investigation of Molecular Basis of Artemisinin Toxicity and Resistance

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Artemisinin and its derivatives are among the front line drugs for malarial treatment due to its high efficacy and minimal side effects. In addition, artemisinins are also active against parasites that are resistant to other types of anti-malarial drugs. The mechanisms of action of artemisinin have been proposed to be through a generation of damaging reactive oxygen species as well as through an inhibition of sarcoplasmic/ endoplasmic reticulum calcium transporter, PfATP6. However, the exact molecular basis of artemisinin-mediated toxicity remains unclear. In addition, recent reports have shown that artemisinin resistance has emerged, although the mechanism of resistance is also unknown. In this study, we utilized baker's yeast Saccharomyces cerevisiae as a model to study the mechanism of artemisinin action and resistance. We found that an alteration in intracellular calcium levels do not affect the toxicity of artemisinin suggesting that an inhibition of transporter protein for calcium may not be a major mechanism of artemisinin toxicity. In addition, artemisinin toxicity appears not to be involved with ER stress, as exposure to toxic concentration of artemisinin does not result in an induction of ER stress or unfolded protein response. We also revealed that cells lacking histone deacetylase Rpd3p show a significant increase in sensitivity to artemisinin suggesting the possible involvement of epigenetic alteration in artemisinin resistance. Our data suggested that Rpd3p-dependent response to artemisinin was mediated through multidrug resistance system.

บทคัดย่อ

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ชื่อโครงการ: โครงการการศึกษากลไกการออกฤทธิ์และกลไกการดื้อยา artemisinin

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ระยะเวลาโครงการ: 2 ปี

Keywords : Artemisinin, แคลเซียม, ภาวะเครียดออกซิเดชัน, Histone deacetylase

ยา artemisin และอนุพันธ์เป็นหนึ่งในกลุ่มยาที่ใช้ในการรักษาโรคมาลาเรียในลำดับต้นๆ เนื่องการประสิทธิภาพในการรักษาที่สูงและการที่ยานั้นมีผลข้างเคียงที่ต่ำเมื่อเทียบกับยารักษาโรคมาลาเรียกลุ่มอื่นๆ นอกจากนั้นแล้ว artemisinin ยังสามารถรักษาโรคมาลาเรียในผู้ป่วยที่ติดเชื้อดื้อยารักษาโรคมาลาเรียอื่นได้อีกด้วย จนถึงปัจจุบันกลไลการออกฤทธิ์ของยา artemisinin นั้นยังไม่เป็นที่ชัดเจน ซึ่งมีการเสนอความเป็นไปได้ของกลไกหลักอยู่ 2 กลไกหลักคือ 1) การก่อให้เกิดภาวะการสะสมอนุมูลอิสระภายในเซลล์ และ 2) การที่ยาไปยับยั้งการทำงานของโปรตีนขนถ่ายแคลเซียมบน endoplasmic reticulum (PfATP6) และเมื่อไม่นานนี้ได้มีการค้นพบเชื้อมาลาเรียที่สามารถดื้อต่อยา artemisinin ได้แต่ทั้งนี้กลไกของการที่เชื้อดื้อต่อยา artemisinin ได้นั้นก็ยังไม่เป็นที่เข้าใจแน่ชัดเช่นกัน ซึ่งในงานวิจัยนี้ได้ทำการศึกษากลไกของการออกฤทธิ์และกลไกการดื้อต่อยา artemisinin โดยใช้ยีสต์ *Saccharomyces cerevisiae* เป็นโมเดลในการศึกษาและพบว่าการเปลี่ยนแปลงของระดับแคลเซียมภายในเซลล์ไม่มีผลกระทบต่อประสิทธิภาพของยา artemisinin และ ยา artemisinin ไม่ได้ก่อให้เกิดภาวะเครียดเนื่องจากความบกพร่องในการม้วนพับของโปรตีนในเซลล์ (ER stress) ซึ่งชี้ให้เห็นว่าการยับยั้งการทำงานของโปรตีนขนถ่ายแคลเซียม PfATP6 ไม่น่าจะเป็นกลไกหลักของการออกฤทธิ์ของยา artemisinin ในการศึกษานี้ยังพบว่าการขาดโปรตีน histone deacetylase Rpd3p ทำให้เซลล์มีภาวะความไวต่อความเป็นพิษของยา artemisinin อย่างชัดเจน แสดงให้เห็นถึงความเป็นไปได้ของผลกระทบจากการเปลี่ยนแปลงทาง epigenetics ต่อการดื้อยาของเชื้อมาลาเรีย ทั้งนี้งานวิจัยนี้ยังแสดงให้เห็นว่ากลไลการเปลี่ยนแปลงดังกล่าวอาจเกิดจากการไปมีผลกระทบต่อระบบการดื้อยาของเชื้อแบบหลายขนาน (multidrug resistance system) โดยตรง

Currently the mechanism through which artemisinin, a widely used anti-malarial drug, exerts its toxic properties is not clearly understood. Several potential mechanisms have been proposed including artemisinin inactivation of a calcium ATPase PfATP6 in the endoplasmic reticulum and mitochondrial damage from increased production of reactive oxygen species (1-3). However, whether there is a link between these potential mechanisms of artemisinin toxicity is not known. In addition, the development of artemisinin resistance in parasites is an increasing problem that is made more difficult due to the lack of a clear understanding of the mechanism of artemisinin toxicity. Genes that are known to be involved in enhancing resistance to other anti-malarial drugs do not appear to be involved in the development of artemisinin resistance making the identification of the molecular pathways leading to resistance more difficult (4, 5). The use of model systems, such as the yeast *S. cerevisiae*, should allow for better understanding of the basic processes involved in artemisinin toxicity and in the development of resistance to this key anti-malarial drug.

Research objectives

Objective 1: To Investigate the molecular pathways of artemisinin toxicity

A number of studies have shown previously that mode of action of artemisinin is through two major pathways, namely an inhibition of PfATP6, a calcium-dependent P-type ATPase and generation of damaging reactive oxygen species (ROS) (3, 6-9). However, the precise mechanisms that lead to the anti-malarial activity of artemisinin remain unclear. We propose to utilize a combination of biochemical and genetic techniques to

investigate the molecular basis of artemisinin-mediated toxicity using the baker's yeast *Saccharoymces cerevisiae* as a model system.

Objective 2: To Investigate of the molecular mechanisms of artemisinin resistance

Many recent studies have provided evidence that artemisinin resistance has already emerged in *P. falciparum* (10-12). Thus, understanding how cells develop resistance to artemisinin is necessary in order to both identify molecular markers to detect resistant parasites as well as to develop novel therapeutic strategies to overcome the challenge of increased artemisinin resistance.

Results and Discussions

Artemisinin toxicity appears independent of intracellular calcium status

It has been suggested that PfATP6, a P. falciparum orthologue of sarcoplasmic reticulum Ca^{2+}-transporting ATPase (SERCA), is a potential target of artemisinin (3) and interestingly, it has been shown in vivo that treatment of *P. falciparum* with artesunate, an artemisinin derivative, results in an increased free cytosolic calcium (13). The proper regulation of calcium homeostasis is essential for many basic cellular processes in Plasmodium falciparum including life cycle, motility, protein secretion, and development (see (14) for review). We investigated if the rise in cytosolic calcium level leads to an increased cellular toxicity caused by artemisinin by using yeast as a model organism. First, we depleted cellular calcium by utilizing an extracellular calcium chelator, EGTA and observed if this can alleviate artemisinin-mediated toxicity.

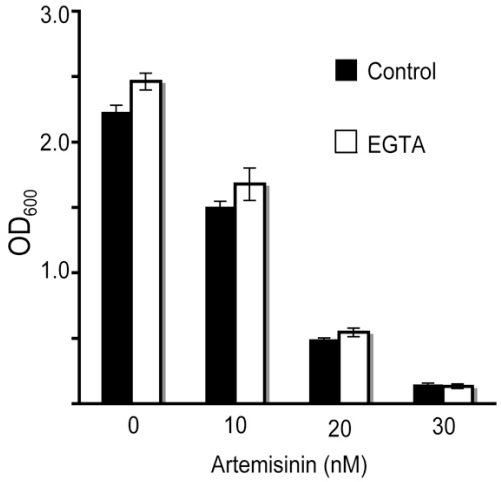

Figure 1. *Reduction of calcium by an extracellular chelator does not appear to alter cellular toxicity of artemisinin in yeast.* Wild-type (BY4742) cells were cultured in yeast peptone supplemented with 2% ethanol and 2% glycerol as carbon sources (YPGE) without an addition of EGTA (black bar) or with an addition of 100 μM EGTA (white bar) prior to culturing the cells with different concentrations of artemisinin. Growth was measured at OD_{600} after 24 hour incubation. The results are a representative of three independent experiments.

The concentration of EGTA used in this study is 100 μM as it is the maximum concentration of EGTA that does not affect growth under the experimental conditions (data not shown). As seen in Figure 1, depletion of calcium does not result in a reduction of artemisinin toxicity. However, it is possible that the concentration of EGTA utilized in this study is not sufficient to significantly reduce a calcium pool inside of the cells. Further analysis on the intracellular level of calcium of the cells under these conditions will be needed to draw a strong conclusion regarding the relationship between the calcium level and the toxicity of artemisinin. Nonetheless, the toxicity of artemisinin is also not altered when we supply the cells with surplus level of calcium (Figure 2).

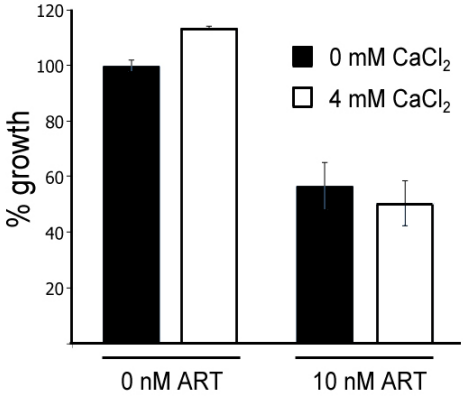

Figure 2. *Artemisinin toxicity was not altered by an increased level of extracellular calcium*. Wild-type (BY4742) cells were cultured in yeast peptone supplemented with 2% ethanol and 2% glycerol as carbon sources (YPGE) without an addition of $CaCl_2$ (black bar) or with an addition of 4 mM $CaCl_2$ (white bar). The toxicity of artemisinin was evaluated by an addition of 10nM artemisnin (ART) to the culture medium. Growth was measured at OD_{600} after 24-hour incubation and was calculated as percentage of growth compared to the control group without addition of calcium and artemisin. The results are a representative of three independent experiments.

In addition, we also investigate if the alteration in an intracellular calcium levels is a possible mechanism of artemisinin-induced cell death by using a genetic approach to manipulate cellular calcium levels. Cells lacking proteins involved in plasma membrane calcium transport; Cch1p, Mid1p, or Ecm7p (15-18) were tested. It has been previously shown that cells deficient Cch1p, Mid1p, or Ecm7p fail to accumulate intracellular calcium in response to stimuli, such as high pH (19).

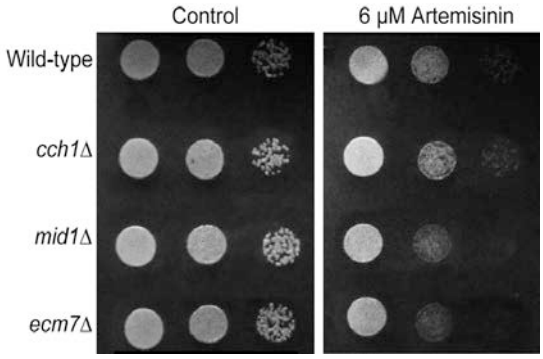

Figure 3. *mid1Δ and ecm7Δ strains shows only slight increased sensitivity to artemisinin.* The yeast wild-type BY4742 cells and the *cch1Δ*, *mid1Δ*, and *ecm7Δ* mutants were tested for growth on the plate with or without 6 μM artemisinin. Approximately 10^4, 10^3, and 10^2 cells were spotted on the plates containing synthetic complete medium supplemented with 2% glycerol and 2% ethanol as carbon sources (SCGE) with and without an addition of artemisinin as indicated and were allowed to grow for 4 days.

As seen in Figure 3, yeast mutants lacking Ecm7p or Mid1p show only a slight increased sensitivity to artemisinin when compared to the wild-type cells suggesting that blocking of calcium uptake does not alter artemisinin toxicity. Consistent with the previous data, this observation suggests the independency between cellular calcium status and artemisinin toxicity.

ER calcium levels were not altered in response to artemisinin toxicity

The possible role of artemisinin as an inhibitor of the ER calcium transporting P-type ATPase PfATP6 suggest a specific effect on ER function may be involved in mediating artemisinin toxicity. Calcium depletion in the ER is known to limit the activity of several molecular chaperones and can result in the accumulation of unfolded proteins within this organelle with a subsequent activation of the unfolded protein response (UPR) to increase expression of ER molecular chaperones and decrease the level of protein translation (20). To monitor if ER stress was involved with artemisinin toxicity, we examined the activity of the unfolded protein response (UPR) using a *UPRE-lacZ* reporter containing the *lacZ* gene regulated by the UPR element (21, 22). As shown in Figure 4, artemisinin exposure did not induce the expression of the *UPRE-lacZ* reporter even at the concentration of artemisinin that cause approximately 40% growth inhibition indicating that

the UPR pathway was not triggered by artemisinin. As a control we tested the effect of dithiothreitol (DTT), a known inducer of the UPR (23, 24), and observed a strong induced expression from the *UPRE-lacZ* confirming that expression from this reporter plasmid was sensitive to induction of the UPR pathway.

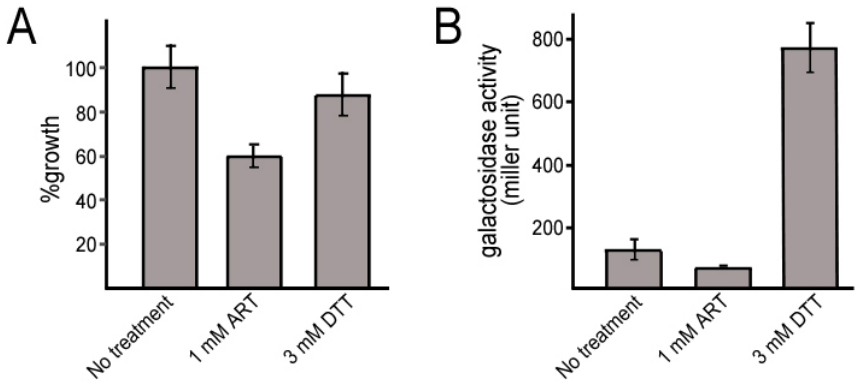

Figure 4. *Artemisinin toxicity does not lead to an induction of ER stress response.* The wild-type strain (BY4742) was grown in the SC-URA media for 12 hours and subsequently transferred to YPD media at an OD_{600} of 0.5 with 1 mM artemisinin, 3 mM DTT or no treatment for 14 hours. The growth of cells measured at OD_{600} (A) and the activity of β-galactosidase from the UPRE-LacZ reporter (B) were monitored.

Pmr1p, the yeast homologue of PfATP6, is known to exhibit an almost total loss of glycosylation of invertase due to low activity of the mannosyl transferase enzymes located in the Golgi (25, 26). We investigated whether cells lacking Pmr1p display increased sensitivity to artemisin and whether an addition of artemisinin results in decreased activity of mannosyl transferase enzymes due to an inhibition of Pmr1p. Artemisinin toxicity in wild-type and *pmr1*Δ cells were not significantly different, though the growth of *pmr1*Δ cells was impaired even under non-stress conditions making comparisons difficult (Figure 5A). We did observe a clear difference in invertase glycosylation in wild-type cells and *pmr1*Δ mutants as had been reported previously (25,

26). As demonstrated in Figure 5B, in contrast to loss of Pmr1p, artemisinin exposure did not alter invertase glycosylation as invertase secreted from wild-type cells exposed to toxic concentration of artemisinin displays high-molecular mass due to glycosylation similar to what observed in wild-type cells without an addition of artemisinin. This result indicates that mannosyl transferase enzymes are not inhibited by artemisinin. Overall, the results we observed from this study do not support a connection between artemisinin toxicity and altered calcium homeostasis or defects in ER function and an inhibition PfATP6 may not be a major mechanism of artemisinin-induced cell death.

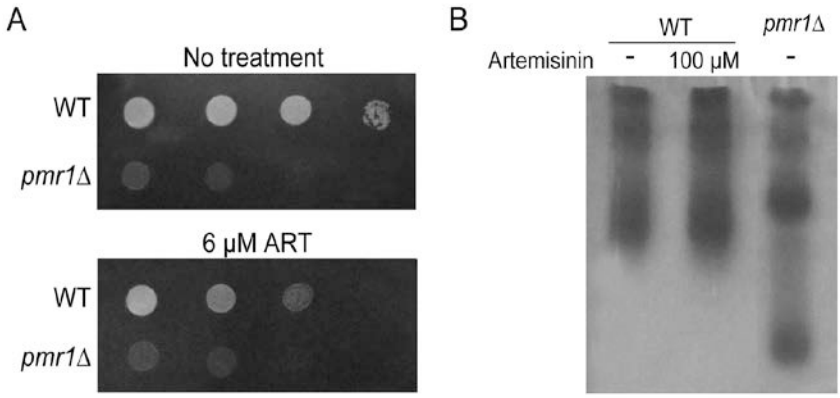

Figure 5. *Artemisinin toxicity does not result in decreased invertase glycosylation and was not altered by loss of Pmr1p*. (A) The yeast wild-type BY4742 cells and the mutants *pmr1*Δ were tested for growth on the plate with or without 6 μM artemisinin. Approximately 10^5, 10^4, 10^3, and 10^2 cells were spotted on the SCGE plates with and without an addition of artemisinin as indicated and were allowed to grow for 4 days. (B) The cell extracts of wild-type cells cultured with and without an addition of artemisinin and *pmr1*Δ mutant cells were analyzed for a glycosylated invertase on the native polyacrylamide gel describe previously (25).

As the results above suggest the the calcium-independent mechanism of artemisinin toxicity, therefore the main mechanism of artemisinin-induced cell detah is likely due to increased cellular oxidative stress. Artemisinin is activated inside of cells

yielding radical intermediates (1, 27) and many studies have suggested that exposure to artemisinin triggers cellular oxidative stress, although the nature of reactive oxygen species generated remains unclear (7, 8, 28-30). Artemisinin has been shown to cause a mitochondrial depolarization *in vivo* and increased ROS was found in isolated mitochondria from parasites treated with artemisinin (7, 8), suggesting that artemisinin toxicity may be mediated through the generation of mitochondrial ROS. However, it is uncertain whether ROS generation mediated by artemisinin is localized exclusively or predominantly in the mitochondrial compartment. We monitored the levels pf protein carbonylation in cells exposed to artemisinin and our preliminary result suggests increased levels of oxidized proteins predominantly in the mitochondria fraction suggesting the possibility that mitochondrial ROS may play a major role in artemisinin-induced toxicity (Figure 6).

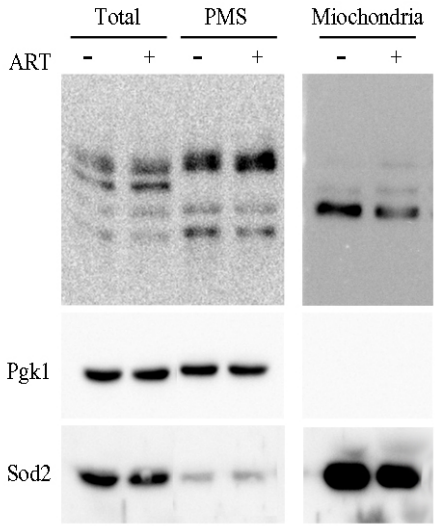

Figure 6. *Increased mitochondria oxidative damage is observed in cells exposed to artemisinin.* Wild-type BY4742 were grown in YPGE to an OD_{600} of 1 and subsequently treated with 2μM artemisinin (+) or vehicle control (-) for 13 hours. Cells were fractionated into the cytosolic post-mitochondrial supernatant (PMS) and mitochondria. 15 μg of protein was reacted with dinitrophenylhydrazine (DNPH), separated by SDS-PAGE, transferred to nitrocellulose, and probed with an anti-DNP antibody (top panels). Pgk1p

and Sod2p were detected as loading controls for cytosolic fraction and mitochondrial fraction accordingly.

ROS signals originating from mitochondria can propagate to other cellular compartments (31). It has been shown that ROS can inhibit the calcium transport activity of SERCA (32, 33) raising the possibility that the inhibition of PfATP6 by artemisinin is due to ROS propagated from mitochondria.

A genetic screen to identify genes that when over-expressed can rescue cells from artemisinin-mediated death

In order to identify genes that can contribute to artemisinin resistance when over-expressed, we performed a genetic selection using yeast multi-copy suppressor analysis. It was found that standard SC Glycerol agar medium was not compatible with selecting yeast colonies directly from the transformation reactions. However, these same transformation reactions produced yeast colonies when plated onto SC medium containing glucose. Since glycerol is not a favored carbon source for *S. cerevisiae* we suspected that transformed yeast cells may be unable to adapt to standard SC Glycerol medium before aborting. Extracellular pH is one factor that influences the growth of yeast and acidification of the medium can be observed during growth on glucose (34). The extracellular acidity is thought to arise naturally from respiration through the production of organic acids (35) and this natural acidification of the medium promotes more rapid growth of yeast cells (36). We later found that adjusting the pH of SC Glycerol medium to 5 would promote colony formation and therefore all subsequent experiments SC Glycerol medium was buffered to pH 5.

Artemisinin was then titrated to find conditions that were toxic to WT yeast cells but able to support the growth of yeast containing a mutation in NDE1 (*nde1*Δ) that is known to display artemisinin resistance (8). The rationale for selecting a concentration of artemisinin that was toxic to WT cells but would allow for growth of the *nde1*Δ mutants was to ensure that the concentration of artemisinin used was not too high and could be overcome by resistant colonies. Addition of 4 μM artemisinin was sufficient to prevent growth of WT yeast while this concentration allowed the *nde1*Δ strain to grow, though at a slower rate than in the absence of artemisinin. Multi-copy suppressors were selected by transforming WT yeast with a YEp13 (*LEU2*) based multi-copy library (37) and selecting for resistant transformants using SC Glycerol medium pH 5 lacking leucine with 4 μM artemisinin. After 5 days of growth many transformants appeared suggesting that the selection conditions for the large-scale transformation were not as strict as when the pilot studies were performed. Subsequent screens will require additional testing of selection conditions to allow for tighter selection of resistant clones. However, several colonies displayed enhanced growth compared to the background colonies and these were picked for additional analysis. From this genetic selection approximately 10,000 transformants were screened out of a needed 50,000 to completely cover the library.

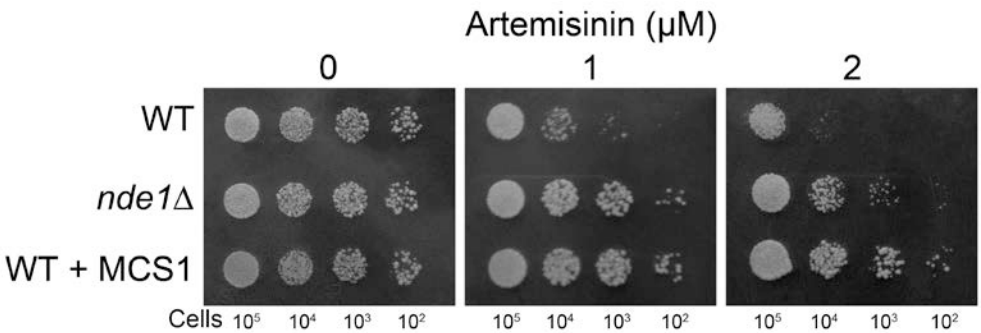

Figure 7. *Identification of a yeast transformant obtained from the screen that displays an increased tolerance to artemisinin.* The yeast BY4742 wild-type and the *nde1*Δ transformants with an empty vector pRS425 and a transformant identified from the genetic screening (WT+MCS) were tested for growth. Approximately 10^5, 10^4, 10^3, and 10^2 cells were spotted on the plates containing synthetic complete (SC) medium supplemented with 3% glycerol with 0μM, 1μM, and 2μM of artemisinin and were allowed to grow for 4 days.

Of the 10,000 transformants 11 colonies appeared to exhibit enhanced growth when challenged with artemisinin. These 11 colonies were tested for artemisinin resistance using a semi-quantitative growth assay and only one displayed enhanced resistance (MCS1) (Figure 7).

We attempted to identify the genes present in the suppressor clone obtained from the genetic screen described above. We discovered that the particular suppressor clone shown in Figure 9 does not contain a suppressor plasmid but instead carrying the secondary mutation on its own genome that allow it to be more resistant to toxicity of artemisinin as re-introducing MCS1 plasmid isolated from the suppressor into the wild-type cells fails to enhance the artemisinin resistance. Further optimizations of the conditions for genetic screen are needed to ensure the recovery of suppressor clone containing the library genes.

Histone deacetylase activity alter cellular sensitivity to artemisinin

It has been shown that an expression of pfHDAC-1, encoding a histone deacetylase enzyme, is significantly decreased in *P. falciparum* resistant to artemisinin

(38). However, whether histone deacetylase activity is directly linked to artemisinin resistance remains unclear. We investigated whether cells lacking histone deacetylase enzymes or the other enzymes involved in chromatin remodeling including histone deacetylases Rpd3p, Sir1-3p, Hda1p, Hos1-4p (39) (40, 41), histone acetyltransferase Sas2p (42), and histone methylase Dot1p (43) would be more tolerant to artemisinin. As displayed in Figure 8, only cells lacking histone deacetylase Rpd3p, which has been shown to have similar sequence similarity to PfHDAC-1, show a significant increase in sensitivity to artemisinin.

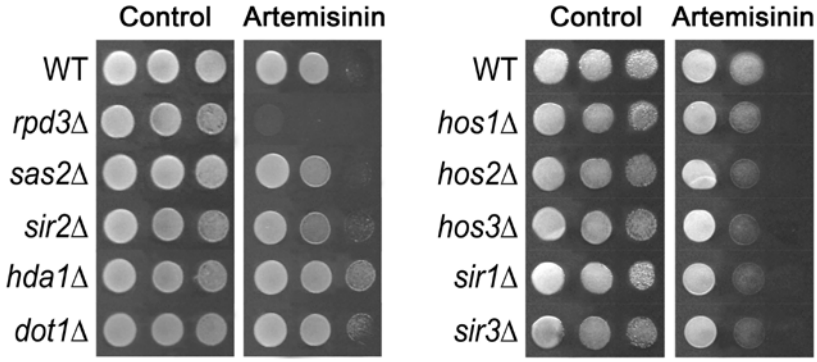

Figure 8. *Mutants lacking Rpd3p histone deacetylase display a significant reduction in cellular tolerance to artemisinin.* The yeast BY4742 wild-type cells and the mutants lacking proteins involved in chromatin remodeling were tested for growth on the solid medium. Approximately 10^5, 10^4, and 10^3 cells were spotted on the plates containing synthetic medium supplemented with 3% glycerol as carbon source (SCG) with and without 3μM artemisinin and were allowed to grow for 4 days.

In yeast, Rpd3p is present as a subunit of two distinct complexes RPD3S and RPD3L (44). These two complexes differ in both their function and composition of subunits (Figure 9A). RPD3S controls histone acetylation patterns and prevents initiation of aberrant cryptic transcription (44, 45), while RPD3L is involved in regulating the

expression of many stress response genes (45-47). We evaluated the role of each RPD3 complex in artemisinin by testing strains lacking key components of each complex and discovered that lacking of subunits of RPD3L (Rtx2p or Pho23p) and not a subunit of RPD3S results in reduced artemisinin tolerance (Figure 9B). The identification of the RPD3L complex shown herein will help to narrow the potential genes that contribute to artemisinin resistance.

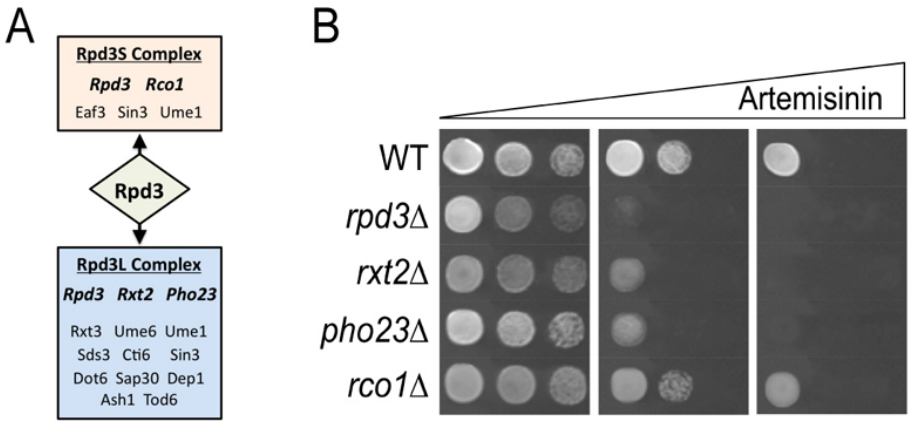

Figure 9. *Artemisinin sensitivity is seen with mutation of subunits from Rpd3L but not Rpd3S.* The yeast BY4742 wild-type cells and the mutants lacking different subunits of RPD3 were tested for growth on the solid medium. Approximately 10^5, 10^4, and 10^3 cells were spotted on the plates containing synthetic medium supplemented with 3% glycerol as carbon source (SCG) with and without 3μM and 6μM artemisinin and were allowed to grow for 4 days.

Interestingly, Rpd3L complex has been shown to have a role in an activation of Msn2/4p, major transcription factors in general stress response of the yeast (48, 49) as well as in modulation of the expressions of pleotropic drug response regulators Pdr1p/Pdr3p (47). We asked if cells lacking Msn2/4p transcription factors would display the reduced tolerance to artemisinin similar to cells lacking a component of Rpd3L.

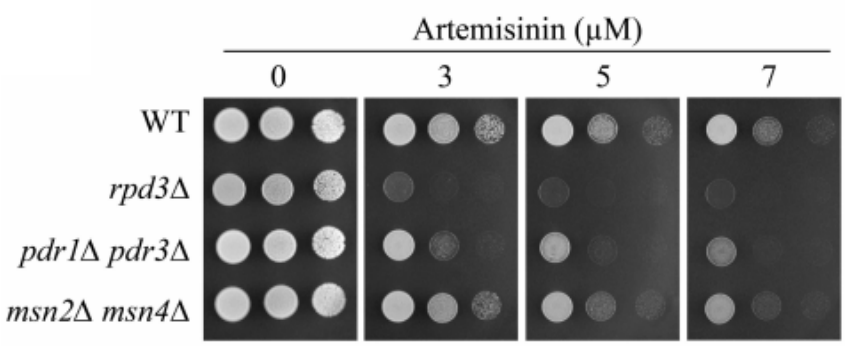

Figure 10. *Lacking of Pdr1p and Pdr3p results in significant decreased tolerance to artemisinin.* The yeast BY4742 wild-type cells and the mutants lacking both Msn2p and Msn4p and both Pdr1p and Pdr3p were tested for growth on the solid medium. Approximately 10^5, 10^4, and 10^3 cells were spotted on the plates containing synthetic medium supplemented with 3% glycerol as carbon source (SCG) with and without 3μM, 5μM, and 7μM artemisinin and were allowed to grow for 4 days.

As shown in Figure 10, Loss of the *MSN2/MSN4* genes caused only a slight increase in artemisinin sensitivity while deletion of *PDR1/PDR3* resulted in more pronounced sensitivity to artemisinin. However, the *pdr1*Δ *pdr3*Δ strain did not exhibited the extreme sensitivity to artemisinin observed in the *rpd3*Δ strain suggesting that addition factors or other modes of regulation are involved in loss of tolerance to artemisinin in cells lacking Rpd3p. Overall, these results suggest that *RPD3* mediated regulation of the PDR system contributes to artemisinin resistance.

Overall, the results present herein suggest; 1) An independency between intracellular calcium levels and artemisinin toxicity, 2) Mitochondria as a major target of artemisinin-induced cell death through an ROS generation, 3) Mechanism of artemisinin resistance through an alteration of pleotropic drug response regulators by a modulation of histone deacetylase activity.

Suggestions for future studies

1) Yeast cells lacking Erg6p, a delta(24)-sterol C-methyltransferase required for ergosterol synthesis (50), may serve as a useful tool for genetic suppressor screening in order to identify genes confer artemisinin resistance when over-expressed. Ergosterol is important to control membrane fluidity and permeability and *erg6*Δ cells have been shown to exhibit enhanced uptake of small molecules (51, 52). Cells lacking *erg6*Δ would anticipate to show increased sensitivity to artemisinin and thus will allow the genetic screen performed by utilizing glucose as a carbon source without using the unusually high dosage of artemisinin. The use of the *erg6*Δ strain background is expected to enhance the genetic library transformation efficiency. This system should allow us to retrieve the number of transformants that can cover the entire genomic library in order to identify other suppressor genes that can rescue cells from artemisinin-mediated cell death.
2) Recent study by Antoine T. and group suggests the iron-mediated mitochondria depolarization as a mode of action of artemisinin (53). In addition, our preliminary results also suggested mitochondria as a major location of ROS generated by artemisinin (Figure 6). Investigating the detailed pathways of how ROS is generated inside the mitochondria would provide more insight into the mechanism of artemisinin-induced cell death and perhaps assist in the development of combination therapy to increase the effectiveness of artemisinin.

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Output จากโครงการวิจัย

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

   ชื่อเรื่อง: Improper trafficking of Pdr5p contributes to artemisinin sensitivity in cells lacking the KDAC Rpd3p.

   ชื่อผู้แต่ง: Amornrat N. Jensen, Worathad Chindaudomsate, Kanate Thitiananpakorn, Skorn Mongkolsuk, Laran T. Jensen

   ชื่อวารสาร: FEBS Letters (submitted)

2. การนำผลงานวิจัยไปใช้ประโยชน์ในเชิงวิชาการ
   1) องค์ความรู้ใหม่ที่เกิดขึ้นเกี่ยวกับกลไกการอออกฤทธิ์และการดื้อต่อยา artemisinin สามารถนำไปศึกษาต่อยอดการศึกษาวิจัยในระดับที่ลึกมากขึ้น
   2) การพัฒนานักศึกษาระดับปริญญาโทในการทำวิจัย (Worathad Chindaudomsate และ Kanate Thitiananpakorn)

3. การเสนอผลงานในที่ประชุมวิชาการ

   Resistance to the anti-malarial drug artemisinin is modulated by the histone deacetylase Rpd3 in *Saccharomyces cerevisiae*. (Oral presentation by Worathad Chindaudomsate) The 39th Congress on Science and Technology of Thailand. BITEC, Bangkok, 2013