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Analysis of the response of *Candida albicans* cells to Silver(I)

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The response of the pathogenic yeast *Candida albicans* to the silver(I) perchlorate salt ($AgClO_4$) was assessed. By employing an anti-phospho-p38 MAPK antibody, dual phosphorylation of a high osmolarity protein (Hog1p) in *C. albicans* in the presence of $AgClO_4$ was demonstrated. Phosphorylation of *C. albicans* Hog1p in response to hydrogen peroxide or $AgClO_4$ resulted in the translocation of this mitogen-activated protein (MAP) kinase to the nucleus. Nuclear translocation of *C. albicans* activating protein-1 (Cap1p) was demonstrated by Western blot analysis and detected using polyclonal anti-Cap1p antibody. Upon $AgClO_4$-induced translocation of Cap1p there was a concomitant activation of genes coding for glutathione reductase-1 and Mn-superoxide dismutase but no increase in the expression of flavin oxidoreductase or mitochondrial processing protease was recorded. In addition, exposure to $AgClO_4$ increased the activity of superoxide dismutase, glutathione reductase and catalase. The activation of *C. albicans* oxidative stress response genes and enzymes following exposure to $AgClO_4$ is evidence of the generation of oxidative stress within this medically important yeast.

Keywords antifungal, Candida albicans, oxidative stress in yeast, silver(I), Hog1p, Cap1p

Introduction

Infections caused by members of the genus *Candida* range from superficial to life threatening systemic and are the third most common group of pathogens in nosocomial blood stream infections in premature infants with the second highest mortality rate [1,2]. Conventional antifungal therapy involves the use of amphotericin B deoxycholate, liposomal amphotericin B, fluconazole, caspofungin, or a combination of fluconazole and amphotericin B [3] but many *Candida* isolates demonstrate tolerance to at least one of these agents [4] thus prompting the search for novel antifungal agents. Silver(I) compounds demonstrate potent antimicrobial properties and may have some applications as antifungal agents [5–9].

The antimicrobial activity of silver (I) has been known for many centuries and the silver (I) ion is the active agent in many healthcare products such as silver-coated catheters [10] wound dressings [5,11] and burn-treatment creams [12]. Wright *et al*. [9] provided evidence of the antifungal activity of a burn wound dressing coated with silver nitrate ($AgNO_3$) against *Saccharomyces cerevisiae*, *C. albicans*, *C. glabrata* and *C. tropicalis*. Additionally, silver nanoparticles have been incorporated into an antimicrobial gel formulation for topical use which demonstrated potent activity against *Aspergillus niger* and *C. albicans* [13]. A silver-containing hydrofiber dressing and a nano-crystalline silver-containing dressing tested against a *Candida* biofilm model also exhibited promising antifungal activity [14]. While the antifungal activity of silver was first reported in 1973 [15], recent work has provided evidence for the potent *in vitro* anti-*C. albicans* activity of silver-containing compounds [7,8,16–18]. Initial studies into the mode of action of silver (I) revealed that the activity of this metal could be inhibited in various growth media [19] and that aqueous silver(I) ions interfere with electron transport [8] and alter the respiration of *C. albicans* cells [6]. It has been suggested that the interaction of silver(I) and the mitochondrion [6–8] coupled with the generation of lipid

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peroxides [8] is evidence for the generation of free radicals while the ability of silver(I) atoms to act as potential Fenton reagents may also contribute to the antifungal activity of this metal.

Candida albicans possesses a number of elaborate pathways to sense and detoxify cytotoxic free radicals, and numerous anti-oxidant mechanisms to reduce the effects of oxidative stress. Two such pathways are the high-osmolarity protein mitogen-activated protein kinase (Hog1p MAPK) pathway [20] and the *C. albicans* activating protein-1 (Cap1p) pathway [21]. The Hog1p MAP kinase pathway is activated in *C. albicans* in response to free radicals [20], and the AP-1-like transcription factor Cap1p is involved in the response to oxidative stress [22–24]. Cap1p function has been shown to be regulated by a nuclear localization mechanism and oxidant regulated translocation of this transcription factor is also required for tolerance to oxidants such as hydrogen peroxide and diamide detoxification in *C. albicans* [21]. Genes coding for glutathione reductase (*GLR1*), a putative NADH-dependent flavin oxidoreductase (*OYE32*), a mitochondrial processing protease (*MAS1*) and the Mn-superoxide dismutase (*SOD2*) are regulated by the Cap1p transcription factor in *C. albicans* [25] and are critical to the cell's response to oxidative stress.

While the potent anti-*C. albicans* activity of silver(I) has been demonstrated [17,18], with the potential role of oxidative stress in mediating cell death suggested as a possible mode of action [26], our understanding of how *C. albicans* responds to this transition metal is poorly developed. The aim of the work presented here was to establish how *C. albicans* responded to silver(I) in order to give an insight into the mode of action of this medically important metal as it may have application in the treatment of fungal infections.

Materials and methods

Culture conditions

Candida albicans ATCC 10231 was maintained on yeast extract-peptone-dextrose (YEPD), i.e., agar (2% (w/v), bacteriological peptone (Sigma, Ireland), 2% (w/v) glucose (Sigma), 1% (w/v) yeast extract (Sigma) and 2% (w/v) agar (Sigma)). *Candida albicans* cells were cultured in RPMI medium (Sigma) supplemented with 2% (w/v) glucose at 30°C and 200 rpm.

Antifungal susceptibility testing

$AgClO_4$ (Sigma) was dissolved in sterile H_2O and antifungal susceptibility testing was performed with *C. albicans* using RPMI medium (Sigma) supplemented with 2% (w/v) glucose as previously described [27]. Susceptibility testing was carried out in triplicate on three independent occasions and results are presented as the mean ± standard deviation. The MIC of 2.5 μg/ml represents the concentration of $AgClO_4$ required to inhibit cell growth by 50%. For all experiments, cells were exposed to the $AgClO_4$ at concentrations corresponding to MIC value.

Extraction of cytoplasmic and nuclear proteins

Early exponential phase cells (2.5×10^6/ml) were exposed to $AgClO_4$ for the periods indicated, harvested by centrifugation at 3,000 *g* for 5 min and washed twice with sterile phosphate buffered saline (PBS) (5 ml). Cells were lysed using glass beads (5 g) in Lambert's breaks buffer (LBB) (3 ml) (100 mM KCl (Sigma), 3 mM NaCl (Sigma), 4 mM $MgCl_2$ (Sigma) and 10 mM PIPES (Sigma). The following protease inhibitors (30 μg) were used; leupeptin (Sigma), aprotonin (Sigma), tosyl-L-lysyl-chloromethane hydrochloride (Sigma) and pepstatin A (Sigma). The resulting supernatant was recovered, centrifuged (5,000 *g* for 5 min at 4°C) and the supernatant retained. For assessing Hog1p phosphorylation, cells were exposed to $AgClO_4$ and lysed using glass beads in 100 mM Tris-HCl (pH 7.5), 1 mM EDTA, 5 mM dithiothreitol (DTT) (Sigma) containing 30 μg of the above phosphatase_inhibitors and the following phosphotase inhibitors: 10 mM sodium orthovandanate (pH 10) (Sigma), 50 mM sodium fluoride (Sigma), 50 mM ß-glycerol phosphate (Sigma) and 50 mM sodium pyrophosphate (Sigma). Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad, Ireland).

In order to verify the successful isolation of nuclear protein, *C. albicans* nuclear fractions were probed with anti-proliferative cell nuclear antigen (PCNA) primary antibody (1:200) (Sigma) and HRP-conjugated horse anti-mouse secondary IgG (1:200) to ensure purity of the fractions. Pure nuclear fractions were probed using polyclonal anti-Cap1p (a kind gift from Prof. Scott Rowley, The Carver College of Medicine, University of Iowa, USA) while both whole cell protein and nuclear fractions were probed using anti-p38 MAPK antibody and anti-phospho-p38 MAPK (Cell Signaling Technology, Massachusetts, USA) as described [20].

RT-PCR analysis

mRNA was extracted using a Qiagen RNAeasy kit (Qiagen, UK) from early exponential phase cells (2.5×10^6/ml) exposed to $AgClO_4$ (2.5 μg/ml) for selected times and cDNA was generated using the Superscript III First-Strand Synthesis System (Invitrogen, Ireland) with oligo (dT) primers. PCR amplifications of *OYE32* (orf19.3131), *GLR1* (orf19.4147), *MAS1* (orf19.3026) and *SOD2* (orf19.3340) genes were performed using the primers and cycles as described [25] and analysis was performed during the log phase of amplification.

The PCR cycle consisted of: 98°C for 2 min, 94°C for 1 min, 55°C for 1 min, 72°C for 1 min 20 sec, and this cycle was repeated for 35 cycles. Visualization of amplified products was performed using a Syngene Geneflash (Mason Technology, Ireland) and densitometric analysis was carried out using Genetools software (Scie-Plas, UK).

Determination of enzymatic activity

Candida albicans cells were exposed to $AgClO_4$ or to hydrogen peroxide (0.5 mM) for 30 min. Cells (1 g wet weight) were harvested, washed in PBS and resuspended in 4 ml of lysing buffer (4 ml Tris-HCl (100 mM, pH 7.5), 4 ml EDTA (1 mM, Sigma), 100 µl pepstatin A (1 mg/ml, added fresh), 100 µl aprotinin (1 mg/ml, added fresh), 100 µl phenylmethanesulphonylfluoride (1 mM, added fresh), and 5 mM DTT (added fresh). To this, 4 g of acid washed glass beads (size: 425–600 µm, Sigma) were added and the mixture was vortexed for 5 min on ice. Cellular debris, broken cell walls and remaining glass beads were removed by centrifugation (250 *g* for 5 min at 4°C, Eppendorf centrifuge 5417R). The pellets were discarded and the supernatant was used.

For analysis of superoxide dismutase activity, the SOD Assay Kit from Sigma was used in accordance with manufacturer's instructions, using a concentration of 1 µg/µl of protein extract. The SOD activity was calculated by employing a tetrazolium salt, WST-1 that produces a water-soluble formazan dye upon reduction with a superoxide anion. The absorbance at 450 nm was read using a microplate reader (Synergy HT, BioTek Instruments, USA) and the following formula was employed to determine SOD activity:

$$\text{SOD Activity} = \frac{[(A_{blank1} - A_{blank3}) - (A_{sample} - A_{blank2})]}{[(A_{blank1} - A_{blank3})]} \times 100$$

For analysis of the glutathione reductase activity of cells, the method described previously [28] was used. The assay employed the extinction coefficient (ε^{mM}) of 6.22 mM /cm for NADPH. Briefly, fresh protein extracts were prepared as above. The following were placed into a clean 1 ml quartz cuvette; 500 µl of 2 mM oxidized glutathione (Sigma), 400 µl of assay buffer (1 mM EDTA, 100 mM potassium phosphate (Sigma), pH 7.5), 50 µl protein extract (1 µg/µl) and 50 µl of 2 mM NADPH (Sigma) A blank was prepared consisting of all the above except 450 µl assay buffer was used and no sample protein was added. The absorbances were read at 340 nm for 2 min at 20 sec intervals (Cary 100 UV-Visible Spectrophotometer, Varian, Inc., USA). The GLR activity was calculated using the following equation:

$$\text{GLR (units/ µl)} = \frac{\text{(Rate of change of sample – Rate of change of blank)}}{\text{6.22 mM/cm} \times \text{Concentration of protein (µg/µl)}}$$

For the determination of catalase activity, fresh protein extracts were prepared as stated. Protein extract (100 µl, 7 mg/ml) was added to 1.8 ml of 17 mM H_2O_2 in a sterile tube. The mixture was mixed well by pipetting and left at room temperature for 15 min. After this time, the suspension was centrifuged at 10,000 *g* (Eppendorf centrifuge 5417R) for 1 min to stop the reaction. The supernatant was removed and placed in a clean quartz cuvette. The absorbance at 240 nm was obtained on a Beckman DU640 spectrophotometer. A blank consisted of 17 mM H_2O_2.

Statistical analysis of experimental data

All experiments were performed in triplicate on three independent occasions and the results are presented as the mean ± standard deviation. The degree of statistical significance was determined using a student t-test assuming $P < 0.05$.

Results

The C. albicans *MAP Kinase Hog1p is phosphorylated and undergoes nuclear translocation in response to hydrogen peroxide or $AgClO_4$*

Phosphorylation of the *C. albicans* MAP kinase Hog1p has been demonstrated to be indicative of the response of *C. albicans* to oxidative stress [20]. Using an anti-phospho-p38 MAPK antibody directed specifically against the human equivalent of *C. albicans* Hog1p, namely p38, dual phosphorylation of the *C. albicans* Hog1p in the presence of 5 mM H_2O_2 or 2.5 µg/ml $AgClO_4$ was demonstrated. Phosphorylation of *C. albicans* Hog1p was found to occur in response to 5 mM hydrogen peroxide for 0.5 h (Fig. 1). Exposure of cells to $AgClO_4$ for 0.5 h, 1 h and 2 h failed to significantly increase the levels of phosphorylated Hog1p. However, exposure of *C. albicans* cells to $AgClO_4$ for 3 h increased the levels of phosphorylated Hog1p (Fig. 1) while the total level of Hog1p in the cell did not increase. The nuclear translocation of phosphorylated *C. albicans* Hog1p was investigated by isolating nuclei from *C. albicans* cells exposed to either 5 mM H_2O_2 or 2.5 µg/ml $AgClO_4$ and probing PCNA positive fraction (Fig. 2a) with an anti-phospho-p38 MAPK antibody (Fig. 2b). Nuclear translocation of phosphorylated Hog1p upon exposure of cells to either hydrogen peroxide or $AgClO_4$ increased when compared to the control (Fig. 2b).

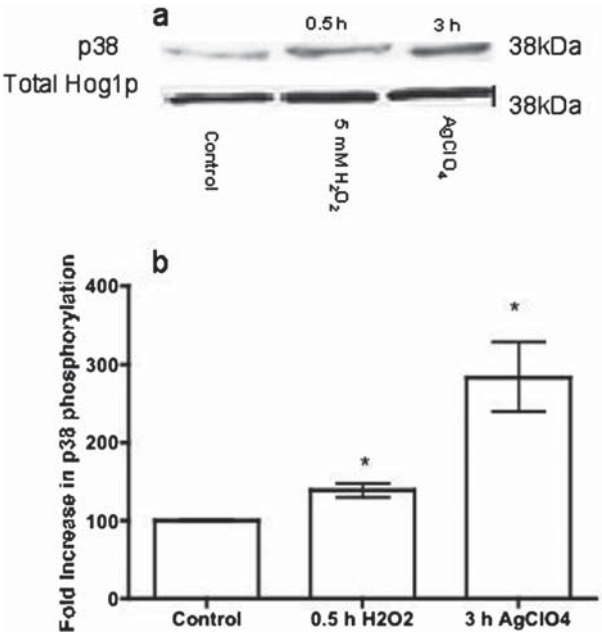

Fig. 1 Exposure of *Candida albicans* to 2.5 μg/ml $AgClO_4$ for 3 h induces p38 phosphorylation. While exposure of cells to 5 mM H_2O_2 for 0.5 h or $AgClO_4$ (2.5 μg/ml) for 3 h increased p38 phosphorylation, the amount of total Hog1p remains constant in all cells. Statistically significant differences (at $P < 0.05$) relative to the control are indicated by (*).

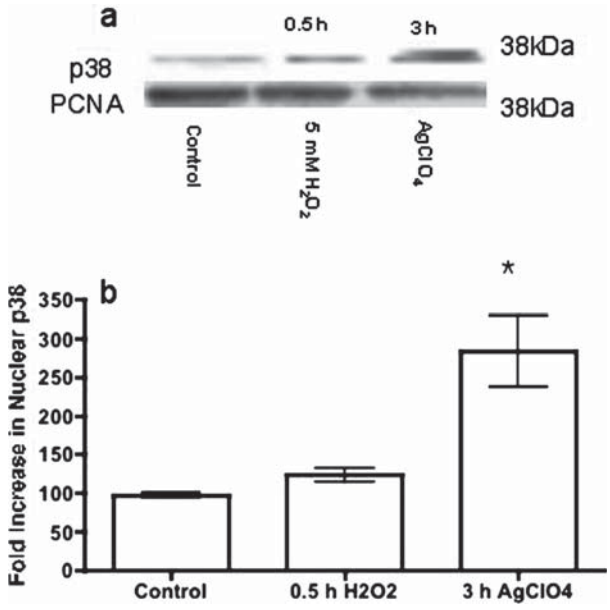

Fig. 2 Induction of p38 nuclear translocation in *Candida albicans* cells exposed to $AgClO_4$ (2.5 μg/ml) for 3 h. PCNA positive fractions were probed for the presence of p38. Exposure of cells to 5 mM H_2O_2 for 0.5 h or 2.5 μg/ml $AgClO_4$ for 3 h increased nuclear translocation of p38. Statistically significant differences (at $P < 0.05$) relative to the control are indicated by (*).

Exposure of C. albicans *to hydrogen peroxide or $AgClO_4$ induces Cap1p nuclear translocation*

Cap1p function has been shown to be regulated by a nuclear localization mechanism and is required for tolerance to oxidants in *C. albicans* [21]. In order to investigate the nuclear translocation of Cap1p, PCNA positive nuclear fractions were probed with polyclonal anti-Cap1p antibody (Fig. 3). For cells exposed to 5 mM H_2O_2 for 0.5 h, no increase in the concentration of Cap1p within the nuclear fraction was observed (Fig. 3a). $AgClO_4$ was found to induce the nuclear translocation of Cap1p after 3 h.

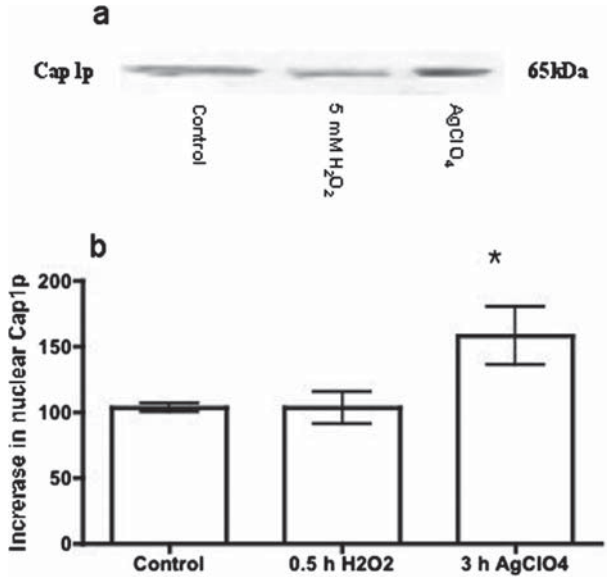

Fig. 3 Exposure of *Candida albicans* to $AgClO_4$ (2.5 μg/ml) for 3 h induces Cap1p nuclear translocation. PCNA positive nuclear fractions were probed with anti-Cap1p antibody. Statistically significant differences (at $P < 0.05$) relative to the control are indicated by (*).

Exposure of C. albicans *to $AgClO_4$ increases the expression of the genes involved in cellular detoxification*

The expression of four of the genes (*OYE32, GLR1, MAS1* and *SOD2*) which Cap1p regulates was investigated following exposure of *C. albicans* to either 5 mM H_2O_2 or 2.5 μg/ml $AgClO_4$ for various periods of time (Fig. 4). No increase in the expression of the *OYE32* gene was observed upon exposure of cells to $AgClO_4$ over 5 h. No significant increase in the expression of the *GLR1* gene was observed over 3 h while a significant increase ($P < 0.05$) in the expression of the *GLR1* gene was observed at 5 h. While no significant increase in the expression of the *MAS1* gene was observed after 2 h, a significant increase in the expression of this gene ($P < 0.05$) was observed after 3 and 5 h. Finally, a significant increase in the expression of the *SOD2*

gene was observed upon exposure of cells to $AgClO_4$ for 5 h (Fig. 4).

Exposure of C. albicans *cells to hydrogen peroxide or $AgClO_4$ increases the activity of detoxifying enzymes*

Previous work has demonstrated that exposure of *C. albicans* to 5 mM hydrogen peroxide results in increased activity of oxidative stress-detoxifying enzymes [20,21]. In the work presented here, determination of the activity of superoxide dismutase (SOD), glutathione reductase (GLR) and catalase (CAT) was performed on *C. albicans* cells which had been exposed to either 5 mM hydrogen peroxide for 0.5 h or to $AgClO_4$ at a concentration of 2.5 μg/ml over 5 h. Total SOD activity increased 125 ± 10% relative to the control when cells were exposed to 5 mM hydrogen peroxide for 0.5 h (Fig. 5). *Candida albicans* cells exposed to $AgClO_4$ demonstrated a significant increase in SOD activity at all time increments greater than 1 h.

Total GLR activity was not significantly increased when cells were exposed to 5 mM hydrogen peroxide for 1 h (Fig. 5). Exposure of cells to $AgClO_4$ increased the activity of glutathione reductase at all times greater than 1 h.

Catalase activity was measured in cells exposed to $AgClO_4$ for different periods of time. The results indicate a significant increase ($P < 0.05$) in activity when cells were exposed to $AgClO_4$ for 0.5–5 h (Fig. 6). Cells exposed to hydrogen peroxide showed a small but statistically significant increase in catalase activity over the time of the experiment.

Discussion

The antimicrobial properties of silver(I) have been exploited recently [5,10–12] and the potent antifungal activity of silver(I) metal is well established although the exact mode of action is still poorly defined [6–8,16–18,26]. Investigations into the mode of action of silver(I) complexes have revealed that this metal modifies the ergosterol content of cells [6], induces apoptosis [7] and alters the mitochondrial cytochrome profile of *C. albicans* [6]. The modification of respiration and the increase in the presence of lipid peroxides in silver(I)-treated cells [8] suggests that the generation of oxidative stress could play a role in the antifungal activity of such silver(I) salts. In this work, the generation of oxidative stress within silver(I)-treated *C. albicans* cells was examined by investigating the activation of the Hog1p MAP kinase and Cap1p pathways.

To investigate whether activation of the *C. albicans* MAP kinase pathway in response to exposure to $AgClO_4$ occurred, the phosphorylation of *C. albicans* Hog1p MAP kinase was examined. No significant increase in Hog1p activation was detected following 2 h exposure to $AgClO_4$, however, it was established that exposure of the yeast to hydrogen peroxide or $AgClO_4$ for 3 h induced Hog1p

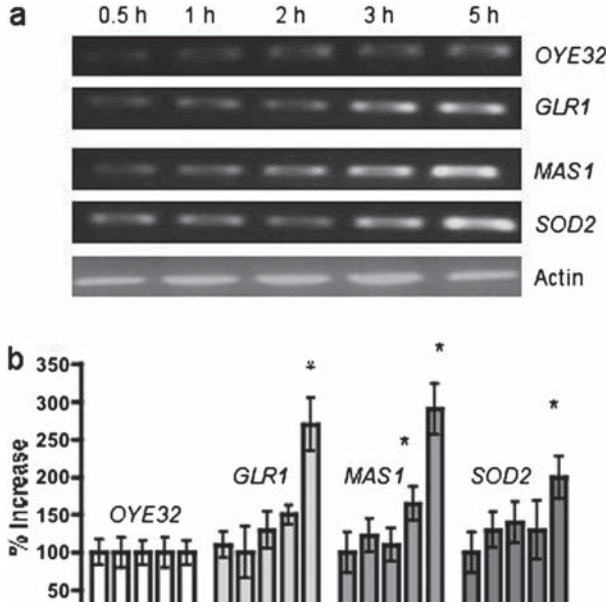

Fig. 4 Exposure of *Candida albicans* to $AgClO_4$ for 0.5 h to 5 h induces expression of genes involved in oxidative stress response. Statistically significant differences (at $P < 0.05$) relative to the control are indicated by (*).

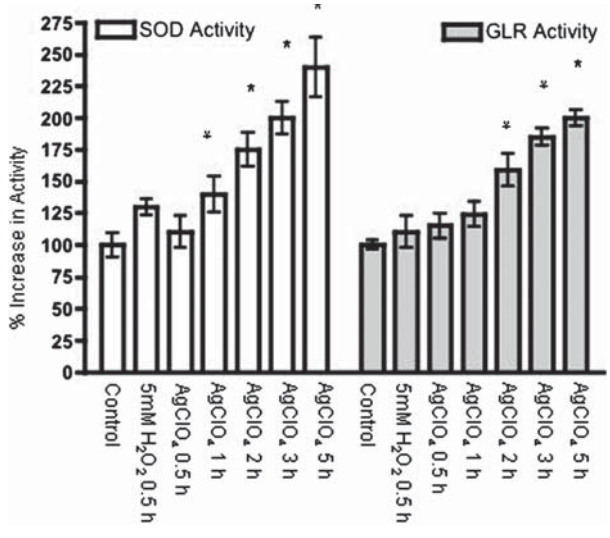

Fig. 5 Exposure of *Candida albicans* to $AgClO_4$ (2.5 μg/ml) over 5 h leads to elevated activity of the superoxide dismutase (SOD) and glutathione reductase (GLR) within *C. albicans*. Differences in the levels of enzyme activity (*) were deemed statistically significant at $P < 0.05$.

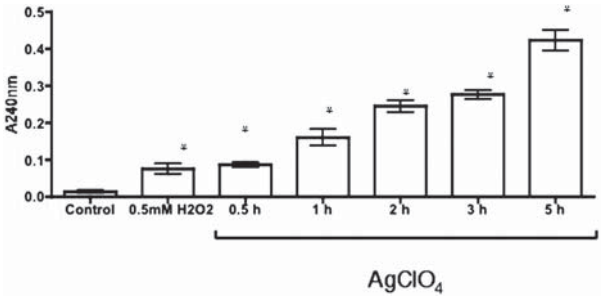

Fig. 6 Catalase activity of *Candida albicans* cells exposed to 0.5 mM H_2O_2 for 0.5 h or $AgClO_4$ for 0.5–5 h. Differences in the levels of catalase activity were deemed statistically significant at $P < 0.05$ (*).

phosphorylation thus providing evidence for the activation of the Hog1p MAP kinase. Once phosphorylated, Hog1p translocates to the nucleus [29], and it was demonstrated that exposure of cells to hydrogen peroxide or $AgClO_4$ induced translocation of the phosphorylated Hog1p to the nucleus thus providing further evidence for the generation of oxidative stress within *C. albicans* upon exposure to $AgClO_4$.

The *C. albicans* Cap1p intracellular signaling pathway is essential in the adaptation of the yeast to oxidative stress [22]. Recently, two dimensional SDS-PAGE analysis of a *C. albicans CAP1* mutant revealed that Cap1p regulates the synthesis of 12 proteins that are responsible for the *C. albicans* response to oxidative stress [30]. Cap1p function has also been shown to be regulated by a nuclear localization mechanism with oxidant regulated translocation of this transcription factor required for normal tolerance to oxidants in *C. albicans* [21]. While exposure of cells to 5 mM H_2O_2 for 0.5 h increased the levels of Cap1p within the nucleus, translocation of this transcription factor was observed to occur in response to $AgClO_4$ after 3 h, thus providing evidence for the generation of oxidative stress within *C. albicans* cells upon exposure to the silver(I) salt.

In addition to the Cap and Hog pathways within *C. albicans*, several other yeasts possess stress responsive MAPK (SAPK) pathways that are induced by oxidative stress. Within *Schizosaccharomyces pombe,* the Spc1 SAPK cascade is induced in response to oxidative stress with activation of this pathway resulting in phosphorylation of the Atf1 transcription factor and an increase in the expression of over 200 genes [31]. Within *S. cerevisiae*, the MAPK Hog1p shares >80% identity with the *S. pombe* Spc1 kinase with Pbs2 and Ssk2, SSk22 and Ste11 acting as the upstream MAPKK and MAPKKK respectively [32]. Furthermore, while the **A**ctivating **P**rotein-1 (AP-1) transcription factor homologue in *C. albicans* is Cap1 [33], the homologue is Pap1 in *S. pombe* [34] with Yap1 acting as the homologue with *S. cerevisiae* [35].

Upon nuclear translocation, the transcription factor Cap1p increases the expression of a number of genes in order to combat the effects of oxidative stress [25]. The expression of four of the genes that Cap1p regulates was investigated using RT-PCR and the expression of three of these genes (*GLR1*, *MAS1* and *SOD2*) was found to be altered in response to $AgClO_4$ in a time dependent manner (Fig. 4). The three genes that demonstrated the most dramatic increase in expression were those coding for a glutathione reductase (*GLR1*), *MAS1* and a Mn-containing superoxide dismutase (*SOD2*) (Fig. 4). Interestingly, the *GLR1* and *MAS1* genes were the ones that exhibited the most dramatic increase in expression upon exposure of cells to $AgClO_4$ for 5 h followed by the *SOD2* gene. The activity of superoxide dismutase and glutathione reductase was increased in cells exposed to $AgClO_4$ for 1 to 4 h. However, no significant increase in the activity of these enzymes was detected after 0.5 h. Catalase activity was increased in cells exposed to $AgClO_4$ for 0.5 to 5 h. The elevation in the activities of these enzymes provides additional evidence for the generation of oxidative stress in *C. albicans* cells following exposure to $AgClO_4$.

In addition to the anti-oxidant defences discussed above, yeasts have evolved further strategies employing numerous other enzymes and small antioxidant molecules to prevent cellular damage caused by reactive oxygen species. Such enzymes include glutathione peroxidases (GPXs), thioredoxins (TRXs) and glutaredoxins (GRXs) [36]. Within *S. cerevisiae*, three GPXs are known and include Gpx1, Gpx2 and Gpx3 with the Gpx3 protein displaying the highest *in vitro* activity [37] with a single GPX gene (*GPX1*) been reported to exist in *C. albicans* [38]. TRXs and GRXs act as thiol oxidoreductases with *C. albicans* also containing two TRX genes (*TRX1* and *TRX2*) and three characterized GRX proteins (Grx1, Grx2 and Grx3). Furthermore, *S. cerevisiae* also contains two methionine sulphoxide reductase enzymes (MSRA, MSRB) [39,40] which are involved in the reduction of oxidized methionine residues with small antioxidant molecules such as glutathione (GSH) and ascorbate (Vitamin C) acting as ROS scavengers within yeast.

Upon exposure of mammalian cells to silver nano-particles, decreased levels of reduced glutathione have been observed, however, no significant change in the levels of GPX are observed [41]. While no known interaction between silver and thioredoxins is known to occur, exposure of mammalian cells to silver fails to significantly alter the levels of methionine sulphoxide within cells [42]. Additionally, a gold containing compound has recently been reported to interact with thioredoxin reductase [43].

The data presented here indicate that exposure of *C. albicans* to $AgClO_4$ at a concentration corresponding to the MIC value results in the activation of Cap1p and Hog1p

pathways – two responses previously demonstrated to be activated under conditions of oxidative stress [20,22,25]. Furthermore, the response to *C. albicans* upon exposure to $AgClO_4$ is also similar to the oxidative stress response of *C. albicans* following exposure to caspofungin [44]. Exposure of *C. albicans* to caspofungin induced activation of the Cap1p and Hog1p pathways, an increase in the expression of the *GLR1* and *SOD2* genes and an elevation in the activity of the SOD and GLR enzymes. Exposure of *S. cerevisiae* to several antifungal drugs that inhibit ergosterol biosynthesis was also found to increase the expression of genes involved in the oxidative stress response such as the *SOD1*, *SOD2* and *GLR1* genes [45]. Exposure of *S. cerevisiae* to the antifungal drug Saframycin A was found to elevate expression of several genes also involved in the oxidative stress response such as the *SOD1*, *SOD2*, *TRX1* and *TRX2* genes [46]. It has also been found that the polyol macrolide niphimycin induces oxidative stress within *S. cerevisiae* [47].

While earlier work has demonstrated that $AgClO_4$ induces oxidative stress in mammalian cells [48] and in bacterial cells [49], this work presents the first evidence for the generation of oxidative stress by this metal in yeast. The results presented here demonstrate that *C. albicans* cells respond to $AgClO_4$ by activating an oxidative stress response and provide further insight into the mode of action of silver(I) [5,6,8,10,15]. While silver(I) is currently in use primarily as an antibacterial agent [5,10–12], these findings may facilitate the increased use of this metal as an antifungal agent where its distinct mode of action [6–8,26] may be advantageous particularly in treating recalcitrant infections caused by fungi demonstrating tolerance to conventional antifungal agents.

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