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Susceptibility of *Trichophyton quinckeanum* and *Trichophyton rubrum* to products of oxidative metabolism

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SUMMARY

Two dermatophyte strains, *Trichophyton quinckeanum* and *Trichophyton rubrum*, were highly susceptible to *in vitro* killing by components of the H_2O_2–peroxidase–halide system. Both strains were, however, resistant to relatively high concentrations of reagent H_2O_2 or H_2O_2 enzymatically generated by glucose and glucose oxidase, KI, or lactoperoxidase (LPO) alone. Resistance to hydrogen peroxidase killing was found to be in part due to the presence of endogenous catalase in the fungi; susceptibility was increased by pretreatment of the fungi with a catalase inhibitor. Kinetic studies using small quantities of reagent or enzymatically generated H_2O_2 and LPO–KI showed that the system was lethal for both fungal strains within 1 min. Furthermore, using the glucose–glucose oxidase–LPO–KI system, it was shown that catalase, superoxide dismutase and histidine scavengers of H_2O_2, superoxide anion and singlet oxygen, respectively, prevented the killing of fungus, whereas scavengers of hydroxyl radicals such as benzoate and mannitol had no effect. *T. quinckeanum* was found to contain large quantities of superoxide anion, as judged by the nitroblue-tetrazolium test. Consequently, the xanthine (or hypoxanthine) and xanthine oxidase system in which the main product is superoxide anion had no toxic effect on the fungus. The high sensitivity of dermatophytes to killing by the H_2O_2–peroxidase–halide system active in polymorphonuclear neutrophils and macrophages may account in part for fungal toxicity *in vivo*.

INTRODUCTION

The importance of oxidative products of the respiratory burst on the killing of bacterial, parasitic and fungal organisms has been well established (Rosen & Klebanoff, 1979; Dockrell & Playfair, 1984; Lehrer, 1969). In particular, the Klebanoff system (Klebanoff, 1968) comprising the reaction between hydrogen peroxide, peroxidase (myeloperoxidase) and a halide is regarded as one of the major components of anti-microbial host defence mechanisms. The reaction involves partial reduction of oxygen, resulting in the formation of highly reactive intermediates such as superoxide anion, singlet oxygen, hydroxyl radicals and halide radicals. This system contributes significantly to the anti-microbial activity of polymorphonuclear neutrophils cells which contain a high concentration of myeloperoxidase (MPO) in the cytoplasmic granules. After phagocytosis, MPO is released into the phagosome where it interacts with endogenous hydrogen peroxide and halides. Among the fungi, susceptibility to oxidative metabolites has been reported with *Histoplasma capsulatum* (Howard, 1981), *Candida albicans* (Lehrer & Cline, 1969), *Aspergillus fumigatus* (Diamond & Clark, 1982) and *Blastomyces dermatitidis* (Sugar *et al.*, 1983).

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In dermatophytes, effector mechanisms that contribute to the killing of fungus in the epidermis are poorly understood. Our previous histological studies in mice experimentally infected with *T. quinckeanum* showed a dense infiltration of neutrophils and macrophages in skin before the peak of the infection (Hay, Calderon & Collins, 1983). However, the role of such cells in defence against dermatophytes has not yet been established, although dermatophyte antigens are chemotactic *in vitro* for human neutrophils (Davies & Zaini, 1984a).

In the present study the effect of products of the respiratory burst on *T. quinckeanum* and *T. rubrum* cell replication is examined in an *in vitro* phagocyte-free system.

MATERIALS AND METHODS

Fungi

Dermatophyte strains, *T. quinckeanum* (NCPF 309) from a previously described isolate (Calderon & Hay, 1984), and *T. rubrum* (MRL 84/633) isolated from a male patient, were obtained from the Mycology Reference Laboratory (LSHTM, London). *T. quinckeanum* is also classified as a form of *T. mentagrophytes* but produces the distinctive clinical picture of mouse favus. The strains were maintained on neutral glucose peptone (NGP) slants at 4° and subcultured on 2% malt agar plates at 28° for 2–3 weeks. Single spore (microconidia)

suspensions were prepared by passage of the disrupted mycelial mat in PBS through a nylon mesh (125 μm). Spores were counted in a haemocytometer, where it was routinely observed that no hyphal fragments were present. Spores were germinated in NGP-broth for 16 hr at 28°. During this time the spores elongated from approximately 4 μm to 15 μm to form germlings. No clumping of germlings was observed at this stage when counted in a haemocytometer.

Chemicals and enzymes

Hydrogen peroxide (H_2O_2, 30% w/v) and sodium azide (N_aN_3) were obtained from BDH Chemicals, Poole, Dorset. The following chemicals and enzymes were obtained from Sigma Chemical Co., Poole, Dorset: histidine hydrochloride (used at 10 mM), sodium benzoate (10 mM), mannitol (50 mM), xanthine (sodium salt) and hypoxanthine (prepared in 50 mM KH_2PO_4 buffer, pH 7·8 containing 10^{-4} M EDTA and used at a final concentration of $1{\cdot}5 \times 10^{-4}$ M), *p*-hydroxyphenyl acetic acid and nitroblue-tetrazolium. Also, lactoperoxidase (LPO: 79 U/mg of protein), glucose oxidase (from *Aspergillus niger*, 1400 U/ml), superoxide dismutase (2900 U/mg), xanthine oxidase (from milk at 4 U/ml) and catalase (25,000 U/mg) were used. Catalase was heat-inactivated at 100° for 15 min. Horseradish peroxidase (250 U/mg) was obtained from Miles, Slough, Berks. Enzymes and inhibitors were diluted in phosphate-buffered saline (PBS), 0·15 M, pH 7·2.

Hydrogen peroxide assay

The hydrogen peroxide generated by the glucose-glucose oxidase reaction was measured by the method of Guibault, Brignac & Juneau (1968). Briefly, a test sample of 50 μl was added to 2·95 ml of Kreb's ringer phosphate glucose (5·5 mM) solution containing 100 μg of *P*-hydroxyphenylacetic acid and 6 U of horseradish peroxidase. The reaction product was read in a fluorescence spectrometer at excitation and emission wavelengths of 315 nm and 410 nm, respectively. The assay was calibrated with known quantities of hydrogen peroxide measured spectrophotometrically using the molecular extinction coefficient $\varepsilon_{230} = 0{\cdot}067$ cm/mM (Maehly & Chance, 1954).

Peroxidative system and fungal viability assay

The standard peroxidative system consisted of three components: lactoperoxidase (18 mU/ml), KI used at a final concentration of 5×10^{-5} M, and H_2O_2 (1 μM–100 mM) added as a reagent, or generated *in situ* by glucose and glucose oxidase. The xanthine (5×10^{-4} M) and xanthine oxidase (2·5–20 mU) reaction to generate superoxide anion was also employed. Phosphate-buffered saline was used in all experiments. Controls included reaction mixtures where individual components were omitted. For the assay, a sample of *T. quinckeanum* or *T. rubrum* germlings containing $2{\cdot}5 \times 10^4$ colony-forming units (CFU) was incubated in the presence of peroxidative components and controls at 37° for 2 hr. The total volume was 1·0 ml. All reaction tubes were prepared in triplicate. Spores were washed twice in PBS and resuspended in buffer by vigorous shaking to obtain a single spore suspension. Samples of spores were mixed with liquid NGP–1·5% agar (37°) and plated in small (50 mm) petri-dishes (approximately 250–300 CFU/plate). Plates were incubated at 28° for 3–6 days and colonies of dermatophytes were counted against an illuminated background. Percentage viability was calculated as follows:

$$\% \text{ viability} = \frac{\text{CFU in experimental system}}{\text{CFU in control}} \times 100.$$

Results are expressed as percentage of control values.

Statistics

Standard error was employed as an estimate of variance, and means were compared by Student's two-tailed *t*-test.

RESULTS

Dermatophyte susceptibility to hydrogen peroxide

Germinated spores of *T. quinckeanum* or *T. rubrum* were exposed to various concentrations of H_2O_2 as a reagent, or generated by the glucose (G)–glucose oxidase (GO) reaction. The results in Fig. 1 show that both *T. quinckeanum* and *T. rubrum* species were resistant to concentrations of up to 1 mM

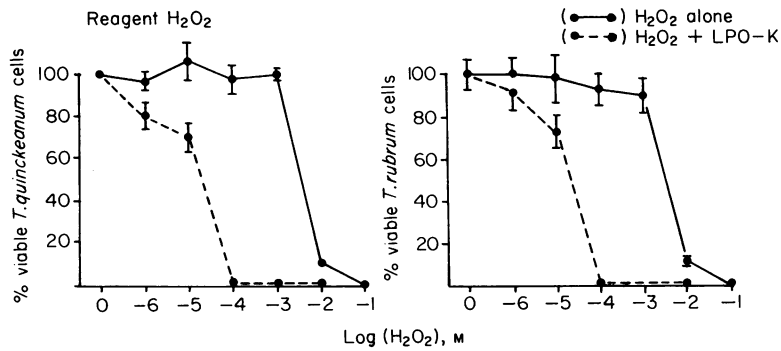

Figure 1. Resistance of dermatophytes to reagent H_2O_2 and susceptibility to H_2O_2–peroxidase–halide. Germlings ($2{\cdot}5 \times 10^4$) of *T. quinckeanum* or *T. rubrum* were exposed to reagent H_2O_2, or a mixture of H_2O_2–lactoperoxidase (18 mU/ml) and 5×10^{-5} M of KI for 2 hr at 37°. Fungal viability was determined by formation of colony units when mixed with NGP–agar in petri-dishes. Results are represented as the percentage of untreated control. Data shown are arithmetic means (±SE) of four separate experiments. Statistical analysis comparing mixture *vs* H_2O_2 alone: $P < 0{\cdot}001$ at 10^3 to 10^{-4} M for both species; $P < 0{\cdot}005$ at 10^{-5} M for *T. quinckeanum*; $P < 0{\cdot}01$ at 10^{-5} M for *T. rubrum*.

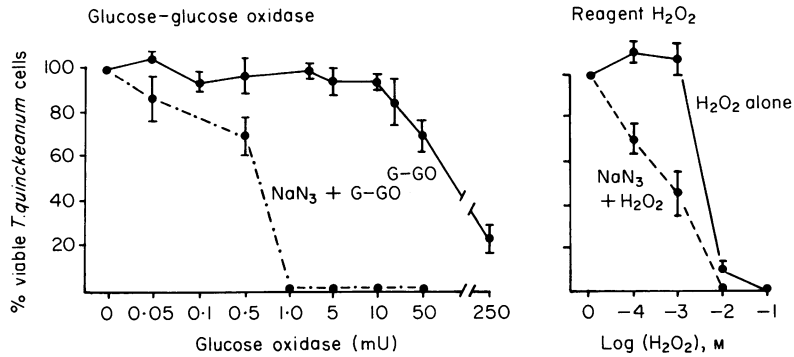

Figure 2. Susceptibility of *T. quinckeanum* to H_2O_2 by pretreatment with sodium azide. Germlings ($2{\cdot}5 \times 10^4$) of fungus were treated with NaN_3 (20 mM) for 1 hr at 37° before the addition of reagent H_2O_2 or glucose (50 mM) and glucose oxidase and then incubated for a further 1 hr. Results represent the arithmetic mean (±SE) of four experiments. Statistical analysis comparing NaN_3 treated *vs* untreated: $P<0{\cdot}005$ at 0·5 mU GO; $P<0{\cdot}02$ at 0·1 mU GO; $P<0{\cdot}001$ at 10^{-3} to 10^{-4} M H_2O_2.

H_2O_2. Similarly, *T. quinckeanum* spores were unaffected by hydrogen peroxide generated by up to 50 mU glucose oxidase (Fig. 2). This amount of GO produced 2 mM of H_2O_2 in 2 hr at 37°, as measured by the method of Guibault *et al.* (1968) (see Materials and Methods). *T. rubrum* spores showed a sensitivity to enzymatically generated H_2O_2 comparable to *T. quinckeanum* (data not shown). During the incubation period with H_2O_2 the production of gas bubbles was observed with the two species tested, suggesting that endogenous catalase might be present. In order to test this possibility the catalase inhibitor sodium azide, which is known to inhibit haem-containing enzymes, was used. Dermatophyte spores were pretreated with NaN_3 (20 mM) for 1 hr at 37° prior to the addition of reagent or G–GO-generated H_2O_2 and incubated for a further 1 hr at 37°. Sodium azide alone had no effect on*T. quinckeanum* viability but it increased the sensitivity of the fungus to H_2O_2 killing (Fig. 2), indicating that endogenous catalase may contribute to resistance to H_2O_2 toxicity. Similar results were obtained with *T. rubrum* (data not shown).

Dermatophyte susceptibility to the peroxidase–H_2O_2–halide system

In order to test whether the dermatophyte species were susceptible to oxidative metabolites generated by the respiratory burst, an *in vitro* cell-free lactoperoxidase (LPO)–H_2O_2–iodide system was used to generate such oxidative products. When germlings of *T. quinckeanum* or *T. rubrum* were incubated with H_2O_2 (10^{-1}–10^{-6} M) in the presence of LPO (18 mU/ml) and potassium iodide (KI, 5×10^{-5} M), the susceptibility to H_2O_2 killing increased by 100-fold from 10 mM to 0·1 mM (Fig. 1). The differences between H_2O_2 alone, or combined with LPO–KI, were significant, with $P<0{\cdot}001$ at 10^{-3}–10^{-4} M for both strains, and $P<0{\cdot}005$ and $P<0{\cdot}01$ at 10^{-5} M for *T. quinckeanum* and *T. rubrum*, respectively. Neither LPO, KI or H_2O_2 alone, nor the combination of LPO–KI, LPO–H_2O_2 or KI–H_2O_2, had a significant effect on fungal viability (Table 1). Moreover, killing of fungi by LPO–H_2O_2–KI was completely abrogated by catalase, but not by heat-inactivated catalase, demonstrating the importance of H_2O_2 in the reaction.

Glucose and glucose oxidase system

The effect of H_2O_2 enzymatically generated by glucose and glucose oxidase was also examined. Fungal spores ($2{\cdot}5 \times 10^4$) were incubated with LPO and KI in the presence of glucose (50 mM) and increasing amounts of glucose oxidase from 0·01 to 10 mU. The amount of H_2O_2 generated by the G–GO reaction at 37° in 2 hr followed a linear relation to the amount of GO. For instance, 0·01 to 10 mU of GO produced 0·4 μM–0·4 mM of hydrogen peroxide, respectively. As can be seen in Fig. 3, almost 50% killing of *T. quinckeanum* spores was achieved with 0·01 mU of GO, and 100% killing with 0·1 mU (4 μm H_2O_2) of GO in the presence of LPO–KI. These results also show that susceptibility of *T. quinckeanum* to the peroxidase–halide system increased when H_2O_2 was enzymatically generated. Total killing was obtained with 100 μM of the reagent H_2O_2 (Fig. 1), but required only 4 μM of generated H_2O_2.

The time–course of killing occurring in this system was determined. It was found that in the presence of LPO–KI over 60% of *T. quinckeanum* cells were killed by 0·05 mU (2 μM H_2O_2)

Table 1. Effect of catalase on the fungicidal activity of LPO–H_2O_2–KI on *T. quinckeanum*

T. quinckeanum treatment*	CFU/plate†	Viability (% control)
None (control)	228±15	100
H_2O_2	219±12	96
KI	226±19	99
LPO	282±20	123
LPO–KI	285±13	125
LPO–H_2O_2	246±21	108
KI–H_2O_2	187±12	82
LPO–H_2O_2–KI	0	0
LPO–H_2O_2–KI + catalase	258±9	113
LPO–H_2O_2–KI + heat-inactivated catalase	0	0

* *T. quinckeanum* germlings ($2{\cdot}5 \times 10^4$) were incubated for 2 hr with LPO (18 mU), H_2O_2 (10^{-4} M), KI (5×10^{-5} M), or combinations as above at 37°. Tubes including catalase (200 μg/ml) or heat-inactivated (100° for 15 min) catalase were incubated with H_2O_2–catalase for 1 hr before the addition of LPO-KI.

† Triplicate tubes, three plates/tube. Results represent the arithmetic mean ± SE of nine plates.

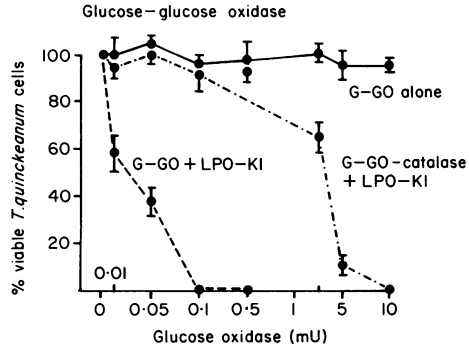

Figure 3. Effect of generated H_2O_2–peroxidase–halide in the presence or absence of catalase on *T. quinckeanum*. Germlings ($2{\cdot}5 \times 10^4$) of fungus were incubated with LPO (18 mU), KI (5×10^{-5} M), glucose (50 mM) and increasing amounts of glucose oxidase for 2 hr at 37°. Alternatively, germlings were incubated with G–GO and catalase (200 μg/ml) for 1 hr before the addition of LPO–KI. Results represent the mean (±SE) of four experiments: $P < 0{\cdot}001$ at all GO concentrations for G–GO + LPO–KI; $P < 0{\cdot}001$ at 2·5 mU GO for G–GO–catalase + LPO–KI.

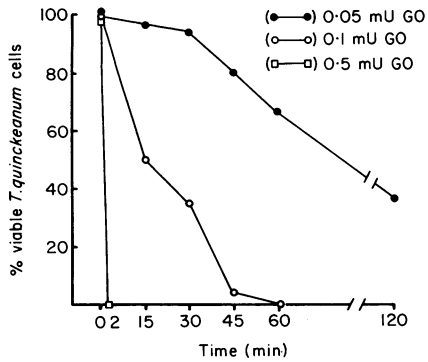

Figure 4. Time–course of killing of *T. quinckeanum* by glucose–glucose oxidase–LPO–KI. A sample of fungus ($2{\cdot}5 \times 10^4$ germlings) was incubated with glucose (50 mM), glucose oxidase, LPO (18 mU) and KI (5×10^{-5} M) at 37°. After incubation, samples were washed twice in PBS, mixed in NGP–agar and plated out in petri-dishes. Results represent the percentage of viable germlings compared to control values.

of GO during the 2 hr incubation period (Fig. 4). Furthermore, increasing GO concentration to 0·5 mU (20 μM H_2O_2) resulted in 100% killing within 2 min, emphasizing once more the potential significance of the peroxidative system in the destruction of dermatophytes.

Effect of O_2 radical scavengers on *T. quinckeanum* killing in the glucose–glucose oxidase system

In order to test which products of the oxidative burst were toxic to dermatophytes, various scavengers of O_2 radicals were used in the G–GO–LPO–KI system. These included catalase (H_2O_2), superoxide dismutase (SOD) (superoxide anion), histidine (singlet oxygen), benzoate and mannitol (hydroxyl radicals). The results are shown in Fig. 5. Catalase, SOD and histidine completely inhibited killing of *T. quinckeanum* by the peroxidase–halide system when GO was used at a concentration of 0·05–0·1 mU (2–4 μM H_2O_2). With higher concentrations of GO

Figure 5. Effect of inhibitors on the killing of *T. quinckeanum* by glucose–glucose oxidase–LPO–KI. Germlings ($2{\cdot}5 \times 10^4$) of the fungus were incubated with G–GO–LPO–KI alone (O---O), or in the presence of benzoate (10 mM), mannitol (50 mM), catalase (200 μg/ml), superoxide dismutase (100 μg/ml), or histidine (10 mM) at 37° for 2 hr. Results represent the mean (±SE) of three experiments.

only catalase inhibited the killing. On the other hand, benzoate and mannitol failed to prevent destruction of fungus by this system. The G–GO reaction is known to generate hydrogen peroxide, but not other oxygen intermediates (Gutteridge, 1980). However *T. quinckeanum* was found to produce superoxide anion which may react with hydrogen peroxide to produce oxygen radicals (McCord & Day, 1978). The production of superoxide anion by *T. quinckeanum* was assessed by the nitroblue-tetrazolium (NBT) dye reduction test (Segal, 1974). Spores (10^6/ml) of *T. quinckeanum* were mixed with an equal volume of a 0·05% solution of NBT, and incubated at room temperature. A black precipitate, indicative of superoxide anion, was formed within 15 min. Furthermore, pretreatment of fungus with SOD inhibited NBT reduction.

Xanthine and xanthine oxidase system

The fungicidal activity of the xanthine (X) or hypoxanthine (HX) and xanthine oxidase (XO) in the presence or absence of LPO–KI was studied. Spores of *T. quinckeanum* were incubated

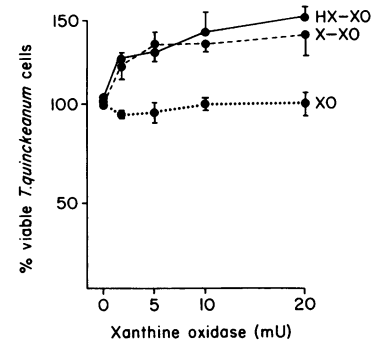

Figure 6. Effect of xanthine, or hypoxanthine and xanthine oxidase on the killing of *T. quinckeanum*. Germlings ($2{\cdot}5 \times 10^4$) of the fungus were incubated with xanthine, or hypoxanthine ($1{\cdot}5 \times 10^{-4}$ M) and xanthine oxidase at 37° for 2 hr. Results represent the mean (±SE) of four experiments. $P < 0{\cdot}001$ for all concentrations of XO *vs* X–XO or HX–XO.

Table 2. Effect of xanthine (hypoxanthine) and xanthine oxidase in the presence of LPO-KI on viability of *T. quinckeanum*

T. quinckeanum treatment*	CFU/plate†	Viability (%control)
None (control)	213±17	100
X	249±22	117
XO	273±9	128¶
X–XO	330±19	155‡
X–XO+SOD	312±22	146‡
X–XO+LPO–KI	312±26	146‡
X–XO+LPO–KI+SOD	304±11	143‡
X–XO+LPO–KI+catalase	318±15	149‡
HX	282±23	132§
HX–XO	292±17	138
HX–XO+LPO–KI	267±15	125¶

* *T. quinckeanum* germlings ($2{\cdot}5\times10^4$) were incubated with xanthine or hypoxanthine ($1{\cdot}5\times10^{-4}$ M) and xanthine oxidase (5 mU) at 37° for 2 hr in the presence or absence of LPO–KI and inhibitors: superoxide dismutase (SOD) 100 μg/ml; catalase 200 μg/ml.

† Results represent the arithmetic mean ± SE of nine plates.

Statistical analysis comparing test *vs* control: ‡ $P<0{\cdot}001$; § $P<0{\cdot}005$; ¶ $P<0{\cdot}01$.

with X or HX and various concentrations of XO (5–20 mU) for 2 hr at 37°. Results are shown in Fig. 6. As can be seen, the viability of *T. quinckeanum* was not only undiminished, but it was apparently increased when germlings were incubated with X (or HX) and XO. Production of superoxide anion, one of the main products of the X–XO reaction, was confirmed by the nitroblue-tetrazolium reduction test. In a subsequent experiment the effect of X–XO in the presence of LPO–KI was examined. The results in Table 2 show that the addition of LPO–KI to the system failed to have a toxic effect on the fungus. Once more, the viability of *T. quinckeanum* was apparently increased in the presence of any component of the system whether alone or in combination.

DISCUSSION

Oxygen intermediates generated by the peroxidase–hydrogen peroxide and halide system were shown to be highly toxic to dermatophytes of the *T. quinckeanum* and *T. rubrum* strains. The LPO–KI system was used as it is the most widely studied alternative to the myeloperoxidase system. The effect of oxygen metabolites on dermatophytes has not been previously investigated. Both strains of dermatophytes under study were strikingly resistant to H_2O_2. For instance, at 10^{-2} M over 90% of fungal spores were still viable (Fig. 1). Endogenous catalase produced by the fungi appeared to be one of the factors contributing to resistance. Using the glucose–glucose oxidase system it was found that susceptibility of dermatophytes to H_2O_2 increased dramatically by pretreating the fungi with sodium azide, a catalase inhibitor. For instance, over 70% of *T. quinckeanum* spores survived exposure to 50 mU (2 mM H_2O_2) of GO, whereas complete fungal killing was achieved with 1 mU (0·04 mM H_2O_2) of GO after treatment with NaN_3 (Fig. 2). However, sodium azide alone did not affect fungal viability.

Susceptibility of dermatophytes to H_2O_2 was also increased by the addition of LPO–KI . In the G–GO system only 4 μM of H_2O_2 were required to kill *T. quinckeanum* completely in the presence of LPO–KI (Fig. 3). This effect occurred in spite of the presence of the endogenous catalase. Furthermore, hydrogen peroxide had a crucial role in the final outcome, since the addition of catalase, but not heat-inactivated catalase, to the system inhibited the toxic activity (Table 1).

Glucose and glucose oxidase is an enzyme system that generates hydrogen peroxide, but not other products (Gutteridge, 1980). Therefore the system *per se* had no fungicidal effect unless LPO and KI were added. The oxygen intermediates produced by the peroxidase–H_2O_2–halide system are hypohalide (e.g. OCI^-) ions, which in turn can react with excess H_2O_2 to produce singlet oxygen and halide ions (Rosen & Klebanoff, 1977). In addition, the dermatophytes' own 'respiratory burst' generates high quantities of superoxide anion. Davies & Zaini (1984b) reported the production of superoxide anion in *T. rubrum*. Here, *T. quinckeanum* was also found to generate superoxide anion. Superoxide may react with H_2O_2 to produce singlet oxygen and hydroxyl radicals (Beauchamp & Fridovich, 1970). In order to test the effect of products of the oxidative burst on dermatophytes, various scavengers were used. It was found that scavengers of hydroxyl radicals such as mannitol and benzoate failed to reverse fungal killing by the G–GO–LPO–KI system (Fig. 5). In contrast, superoxide dismutase and histidine, scavengers of superoxide anion and singlet oxygen respectively, reversed the toxicity. However, this effect was only limited to concentrations of GO between 0·05 and 0·1 mU, equivalent to 2–4 μM of reagent H_2O_2. Catalase, on the other hand, prevented killing in this system at concentrations of hydrogen peroxide ranging from 2 μM to 100 μM (Fig. 3). Thus, these results support a potential role of hypohalide ions, and to a lesser extent singlet oxygen as the toxic mediators. In order to confirm the definitive role of the latter, the possibility that histidine might quench the LPO–H_2O_2–KI oxidant directly would need to be excluded.

The xanthine and xanthine oxidase reaction generates superoxide anion as the main product (Babior, Curnutte & Kipnes, 1975), but subsequently the production of other oxygen metabolites including H_2O_2, hydroxyl radicals and singlet oxygen may ensue (Beauchamp & Fridovich, 1970). However, this system was not inhibitory to dermatophytes, and incubation of *T. quinckeanum* with xanthine and xanthine oxidase produced an unexpected increase in fungal viability (Fig. 6). A similar effect was shown for *T. rubrum* (data not shown). Superoxide anion is a powerful redox reagent capable of undergoing oxidation to O_2 or reduction to H_2O_2 and other oxygen metabolites (Yamazaki & Piette, 1963). Judging by the dramatic increase in fungal viability in the presence of X–XO, one may speculate that the fungus is using the superoxide anion to its own advantage by oxidizing it to O_2, a metabolite required for its survival. This assumption is further confirmed by the fact that the addition of LPO–KI to the system failed to have any effect (Table 2), which indicates that very little, if any, hydrogen peroxide is produced from the excess superoxide anion present in the system.

In summary, two dermatophytes, *T. quinckeanum* and *T. rubrum*, were found to be resistant to hydrogen peroxide, but

their susceptibility improved considerably either by inhibiting endogenous catalase with sodium azide or by the addition of lactoperoxidase and KI. The *in vivo* equivalent, the myeloperoxidase–H_2O_2–halide system, is highly active in polymorphonuclear neutrophils (McRipley & Sbarra, 1967). An investigation into the physiological significance of this system against dermatophytes using human neutrophils and monocytes has subsequently been undertaken (Calderon & Hay, 1987).

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