Parasitol Res (2007) 102:91–97
DOI 10.1007/s00436-007-0729-y

ORIGINAL PAPER

In vitro antileishmanial activity of resveratrol and its hydroxylated analogues against *Leishmania major* promastigotes and amastigotes

Lukasz Kedzierski · Joan M. Curtis · Milena Kaminska · Jadwiga Jodynis-Liebert · Marek Murias

Received: 8 August 2007 / Accepted: 14 August 2007 / Published online: 1 September 2007

Abstract Resveratrol, a natural phytoalexin found mainly in grapes, possesses a variety of beneficial activities including anticancer, antimicrobial and antiviral. However, there is no information about its effects on kinetoplastid parasites such as *Leishmania*. *Leishmania* is a human pathogen responsible for a spectrum of diseases known as leishmaniases and a significant health problem in many parts of the world. In this study, we investigated effects of resveratrol and its hydroxylated analogues on *Leishmania major*, a causative agent of zoonotic cutaneous leishmaniasis in the Old World. Resveratrol showed antileishmanial activity against promastigotes in vitro and, more importantly, was effective against intracellular amastigotes, a parasite life stage infectious in humans, as detected in in vitro macrophage assay. The hydroxylated stilbenes tested in this study also showed antileishmanial activity against promastigotes, the most promising being 3,4,4′,5′-tetrahydroxy-*trans*-stilbene. This compound showed excellent antileishmanial activity against extracellular promastigotes in vitro but not intracellular amastigotes. Our results suggest that resveratrol may be useful as a therapeutic agent to treat leishmaniasis and warrant its further assessment in animal models of disease.

L. Kedzierski · J. M. Curtis
Infection and Immunity Division,
Walter and Eliza Hall Institute of Medical Research,
Parkville 3050 Victoria, Australia

M. Kaminska · J. Jodynis-Liebert · M. Murias
Department of Toxicology,
Poznan University of Medical Sciences,
60-631 Poznan, Poland

L. Kedzierski (✉)
1G Royal Pde.,
Parkville 3050 Victoria, Australia
e-mail: kedzierski@wehi.edu.au

M. Murias (✉)
ul. Dojazd 30,
60-631 Poznan, Poland
e-mail: marek.murias@amp.edu.pl

Introduction

Leishmaniasis is a spectrum of diseases ranging in symptoms from skin lesions to fatal systemic infection caused by protozoan parasites of the *Leishmania* species (Handman 1999). It has a worldwide distribution and currently threatens 350 million individuals in 88 countries around the world. The burden of disease expressed in disability-adjusted life years (DALYs) is estimated to be more than 2.3 million (Desjeux 2004), and WHO has classified leishmaniasis as a category 1 disease, i.e. emerging and uncontrolled (Murray et al. 2005). In the absence of vaccines, chemotherapy is the only means of controlling the disease. Treatment relies on pentavalent antimony, but its use is becoming limited due to the emergence of drug resistance and loss of efficacy. Unfortunately, only a few drugs are available as a second-line therapy in case of antimonial failure, but cost and duration of treatment, unwanted side effects and toxicity hamper their use (Davis and Kedzierski 2005). The experience with various antiparasitic drugs as well as antibiotics indicates that the emergence of resistance is a commonly occurring phenomenon rather than the exception. This prompts the continuous search for new compounds with antiparasitic activity.

One potentially rapid and cost-effective approach to identifying and developing new antileishmanial drugs is screening of existing drugs with good safety profiles and oral bioavailability, already approved for other uses. The latest

antileishmanial drug introduced into the clinical practice is miltefosine (Croft and Engel 2006). It was initially identified and evaluated as an anticancer drug (Eibl and Unger 1990), and its antiprotozoal activity was subsequently demonstrated against visceral leishmaniasis in humans (Jha et al. 1999). One of the compounds characterised by a range of beneficial effects, including anticancer activity, is resveratrol (3,4′,5-trihydroxystilbene). Resveratrol is a natural phytoalexin found in grapes and red wine (Siemann and Creasy 1992). It possesses anticancer, antioxidant and cardioprotective properties, can enhance stress resistance and extend life-span of various organisms (Baur and Sinclair 2006), and is well-tolerated at high doses without adverse effects (Juan et al. 2002). Its activity has been demonstrated against dermatophytes (Chan 2002), parasitic fungi (Leiro et al. 2004b) and viruses (Docherty et al. 1999) Effects of resveratrol on protozoan parasites such as *Leishmania* have not yet been addressed. The vast range of resveratrol bioactivities, its lack of toxicity, and most importantly its activity against skin pathogens prompted us to investigate its effects on *Leishmania major*, a causative agent of cutaneous leishmaniasis. In this study, we have evaluated for the first time the effects of resveratrol and its hydroxylated analogues (Murias et al. 2005) on *L. major* promastigotes and intracellular amastigotes in vitro and assessed the toxicity of these compounds on several cell types.

Materials and methods

Cell culture

L. major V121 (MHOM/IL/67/JERICHO II; WHO Reference Centre for Leishmaniases, Jerusalem, Israel) was maintained at 26°C in M199 medium (Invitrogen) supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS; Trace Biosciences). The murine macrophage J774 (ATCC, Rockville, MD, USA) and myeloma Sp2 (ATCC) cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 10% HI-FBS at 37°C in 5% CO_2. Mouse peritoneal macrophages were harvested and cultured as described (Kelleher et al. 1995). Normal human skin fibroblasts (a gift from Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw) were maintained in DMEM containing 10% HI-FBS, 2 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin at 37°C, in 5% CO_2.

Synthesis of hydroxylated resveratrol analogues

Hydroxylated and methylated resveratrol analogues were synthesised as previously described (Murias et al. 2004). Resveratrol was obtained from Sigma (Munich, Germany).

Cell viability assay

The CellTiter Blue Cell Viability Assay (Promega, Madison, WI, USA) was used for screening for antileishmanial activity and toxicity. Compounds were dissolved in dimethyl sulphoxide (DMSO) at 10 mg/ml working stock and diluted out in appropriate culture media. The assay was set up in triplicates in 96-well plates according to manufacturer's instructions. Approximately 10^6 promastigotes/ml and 10^5 macrophages or Sp2 cells/ml were used. Cell viability was assessed spectrophotometrically at 550 nm with the reference wavelength of 630 nm (Mikus and Steverding 2000). The CellTiter Blue dye was added to samples at the time of setting up the assay, and T_0 value was subtracted from all subsequent readings as a background value. All readings were compared to the no-drug control, and the percent growth inhibition was calculated. DMSO controls were included. All plates were assessed microscopically. The crystal violet assay was used to assess viability of fibroblasts (Mickuviene et al. 2004). Fibroblasts between the fifth and ninth passages were detached by trypsinization and seeded into 96-well plates at the density of 15,000 cells/well in 200 μl culture and used at day 4 when subconfluence was reached. Cells were treated with different compounds for 48 h, then washed with phosphate-buffered saline (PBS), fixed and stained with 0.1% crystal violet in methanol for 10 min. Cells were washed twice with PBS, 100 μl of a 50% glacial acetic acid solution added, and cells were resuspended in plate shaker for 30 min at room temperature. Absorbance was measured at 540 nm with the reference wavelength of 650 nm. All readings were compared to the no-drug control, and the percent growth inhibition was calculated.

Amastigote macrophage assay

Standard macrophage invasion assay in 24-well plates with 5:1 invasion ratio (5×10^5 promastigotes and 10^5 macrophages) was performed as described (Kedzierski et al. 2004). No-drug controls, DMSO controls and no-invasion drug controls were included in each assay. After Giemsa staining, coverslips were examined microscopically and percentage of infected cells counted.

Results

Effects of resveratrol on viability of *L. major* promastigotes and amastigotes in vitro

We first investigated whether resveratrol at 10, 50 or 100 μg/ml affects proliferation of *L. major* promastigotes in

vitro. Parasites were incubated for 48 h in the presence of resveratrol, and the growth rate was compared to that of no-drug control. Controls containing DMSO at concentrations equivalent to those in different resveratrol doses were included and showed no adverse effect on promastigote viability (data not shown). Resveratrol showed antileishmanial activity in a dose-dependent manner and, at 10, 50 and 100 μg/ml, reduced parasite viability by 32, 55.8 and 65.5%, respectively (Fig. 1a). IC50, i.e. the concentration that caused 50% reduction in survival in comparison to no-drug control, was estimated from the graph to be 45 μg/ml. A range of lower resveratrol concentrations (2–40 μg/ml) was tested to assess effects on parasite viability over a period of 144 h. Despite longer incubation period, lower resveratrol doses showed only moderate antiparasite activity (data not shown). Microscopic examination of all samples indicated that the observed effect is leishmanicidal rather than leishmnistatic as the morphology indicated that the promastigotes were dead. Microscopy also confirmed that no-drug controls proliferated at normal rate as indicated by spectrophotometric readings (data not shown). G418 at 10 μg/ml was initially used as a reference compound, and after 68 h incubation all parasites were eliminated from the test sample (data not shown).

The activity of resveratrol on intracellular amastigotes was investigated in a macrophage assay. Mouse peritoneal macrophages were incubated with promastigotes at a ratio of 1:5 for 2 h. After removal of free promastigotes, infected macrophages were incubated for 48 h in the presence of resveratrol at 40 and 45 μg/ml, i.e. a range of concentrations that was estimated to cause 50% reduction in promastigotes in vitro. A significant reduction in the number of infected macrophages was observed for both concentrations (P=0.0003 and P<0.0001), although no significant difference was observed between different doses (Fig. 1b). More importantly, resveratrol significantly (P= 0.001 and 0.003, in two independent experiments, although with markedly different levels of infection) reduced the number of infected macrophages at 20 μg/ml, a concentration that showed only moderate activity on promastigotes in vitro (Fig. 1c). Non-infected macrophages were also incubated in presence of resveratrol at 20, 40 and 45 μg/ml, and the compound had no adverse effects on their viability and morphology (data not shown). This is in agreement with previous reports (Juan et al. 2002) indicating that resveratrol is well-tolerated and non-toxic.

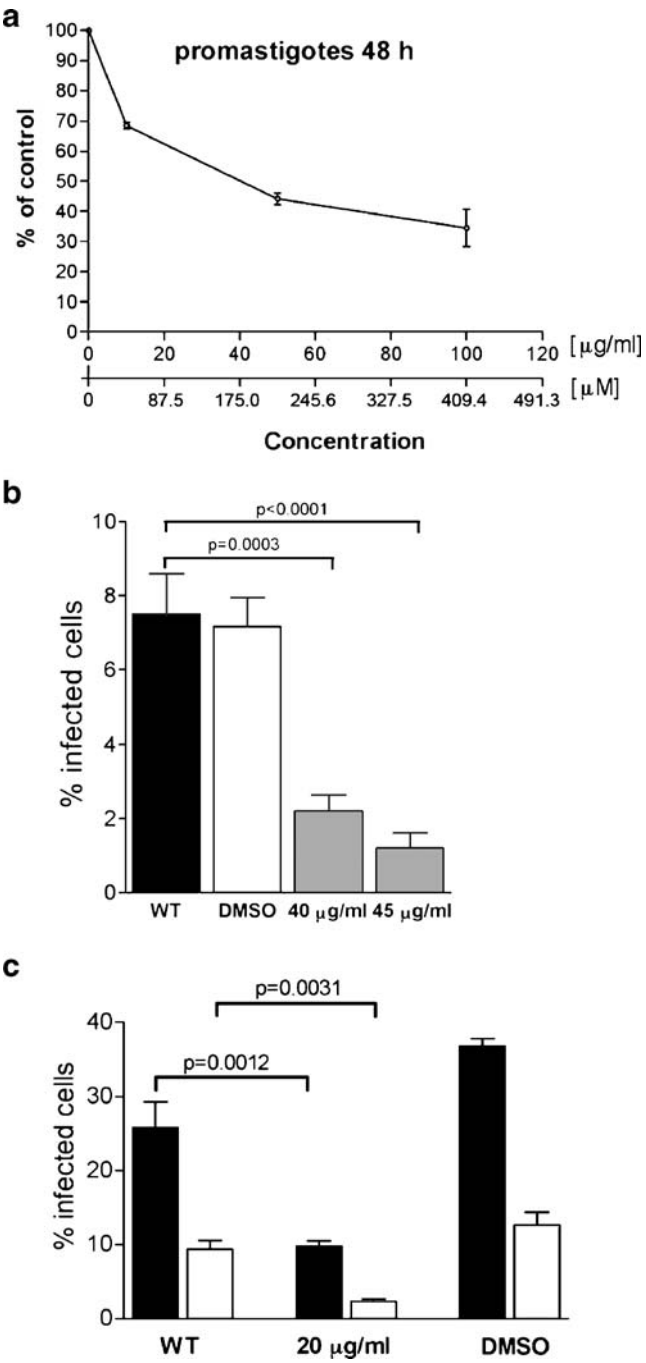

Fig. 1 Effects of resveratrol on *L. major* promastigotes and amastigotes. **a** Dose–response curve for resveratrol tested on promastigotes in vitro. In vitro treatment of *L. major*-infected mouse peritoneal macrophages with resveratrol. **b** Activity of resveratrol at IC50 concentrations. **c** Activity of resveratrol at 20 μg/ml, two independent experiments are shown (*closed and open bars*). *P* values were calculated using non-parametric Mann–Whitney test. *Error bars* represent SEM

Screening of resveratrol analogues for antileishmanial activity

Fifteen methylated and hydroxylated resveratrol analogues were tested for inhibition of growth of *L. major* promastigotes in vitro. All compounds were initially tested at 10, 50 and 100 μg/ml. Methylated derivatives showed poor solubility in culture media and were excluded from testing. After 48 h incubation, all hydroxylated analogues showed antileishmanial activity at 50 and 100 μg/ml (data not shown), and majority showed activity at 10 μg/ml (Fig. 2a). Based on the initial

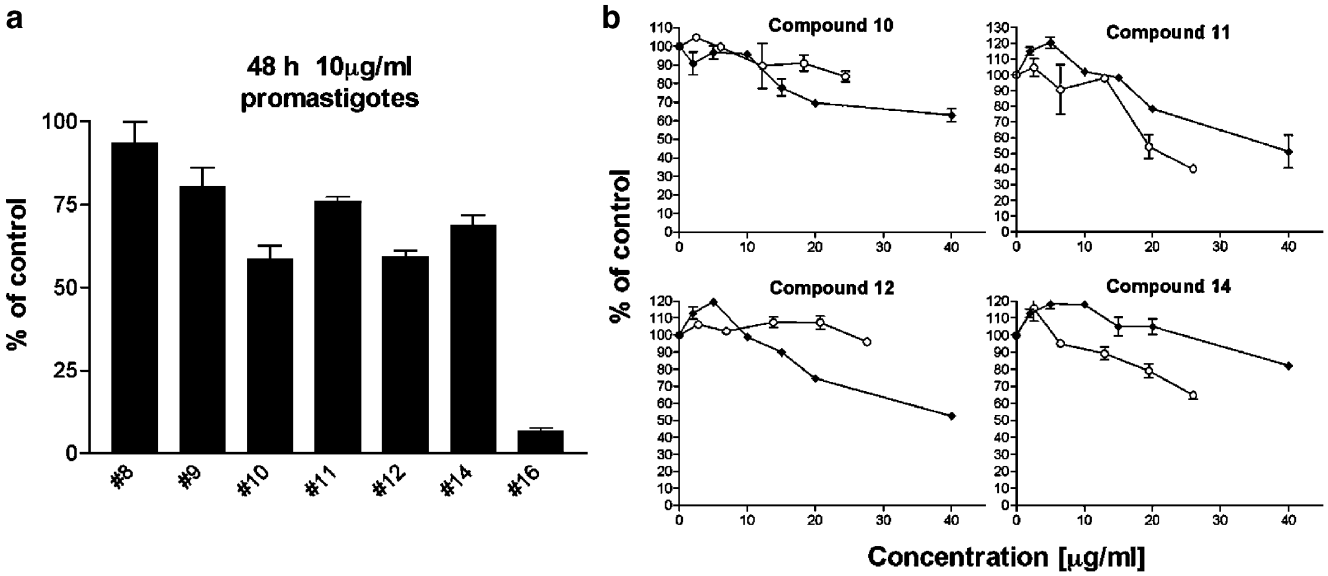

Fig. 2 Antileishmanial activity of hydroxylated resveratrol analogues. **a** *L. major* promastigotes were treated with different compounds at 10 μg/ml for 48 h. Triplicate values were plotted for each compound and expressed as a percentage of no-drug control. **b** Dose–response curves for selected hydroxylated stilbenes. Compounds were tested on *L. major* promastigotes in vitro (*closed symbols*) and fibroblasts (*open symbols*) and cell viability expressed as a percentage of no-drug control. *Error bars* indicate SEM

testing, compounds 8 and 9 showed no significant effect when compared to the no-drug control and were excluded from further testing. The remaining compounds were tested across a range of concentrations (2–40 μg/ml) over 144 h (Fig. 2b). Compound 10 showed moderate leishmanicidal activity at concentrations not toxic to fibroblasts, whereas compound 12 showed activity comparable to that of resveratrol. Conversely, compounds 11 and 14 were toxic to fibroblasts and promastigotes. The most promising compound 12 was tested in a macrophage assay at 40 μg/ml, but no anti-amastigote activity was observed (data not shown).

Determination of antileishmanial potency and toxicity of compound 16

Compound 16 (3,4,4′,5′-tetrahydroxy-*trans*-stilbene) showed the most potent anti-promastigote activity in vitro (Fig. 2). Therefore, we were particularly interested in comprehensive testing of its efficacy and assessment of its toxicity to other cell types. The compound was tested at 1, 2, 5, 10, 20, 30 and 50 μg/ml during 120 h incubation. Significant anti-leishmanial activity was observed after 48 h (Fig. 3a) at 5 μg/ml (20.5 μM) and 10 μg/ml (40.1 μM) that eliminated 32 and 43% of promastigotes (in comparison to no-drug control), respectively. After longer incubation period, compound 16 further reduced numbers of viable promastigotes to 52 and 29% at 5 and 10 μg/ml, respectively. After 144 h incubation, microscopic observation revealed that at 20, 30 and 50 μg/ml, no live parasites could be observed, and at 10 μg/ml, majority of promastigotes were dead (data not

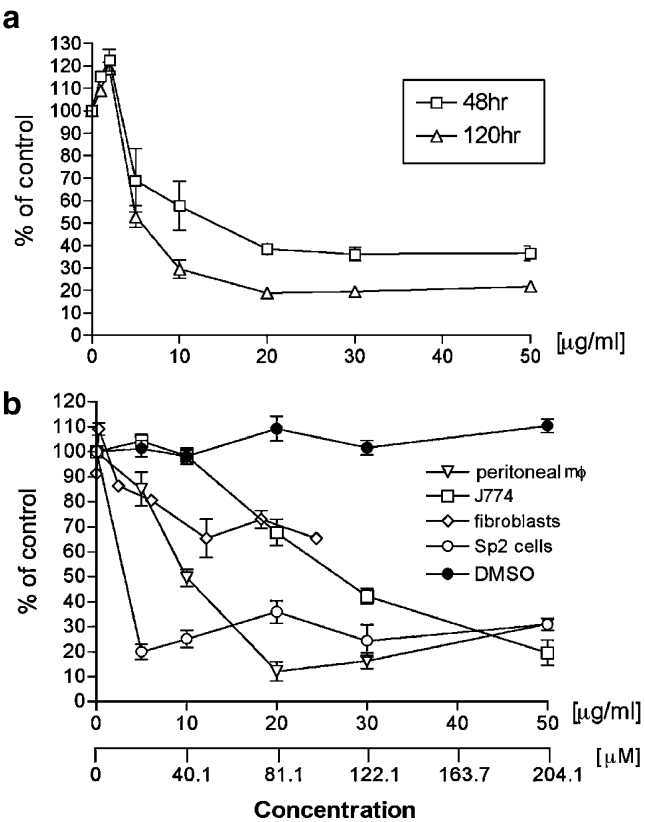

Fig. 3 Effects of 3,4,4′,5′-tetrahydroxy-*trans*-stilbene on *L. major* and host cells. **a** Dose–response curve for 3,4,4′,5′-tetrahydroxy-*trans*-stilbene tested on promastigotes in vitro. Triplicate values were plotted for two time points. **b** Effects of 3,4,4′,5′-tetrahydroxy-*trans*-stilbene on various cell types in vitro, proliferation expressed as a percentage of no-drug control (equivalent DMSO controls were included). *Error bars* indicate SEM

shown). In the macrophage assay, however, no reduction in the number of infected macrophages at either 5 or 10 μg/ml was observed (data not shown).

The effects of compound 16 were also tested on fibroblast, peritoneal macrophages, J774 macrophage cell line and Sp2 myeloid cell line to assess its toxicity to host cells. Two lowest concentrations showing leishmanicidal activity did not affect J774 macrophages after 48 h incubation; however, these had a profound effect on Sp2 myeloid cells, and to a lesser degree on peritoneal macrophages and fibroblasts (Fig. 3b).

Discussion

A plethora of plant-derived polyphenols has been shown to protect tissues against oxidative stress and pathologies such as cancer, coronary heart disease and inflammation (Tapiero et al. 2002). Despite the widely acknowledged protective properties, not much is known about antimicrobial or antiparasitic activities of these compounds. Perhaps the most extensively tested group of polyphenols are water-soluble tannins (Kolodziej and Kiderlen 2005). Conversely, reports on antiparasitic properties of stilbenoids are very scarce. Antiprotozoal activity has been demonstrated for natural stilbenoids (del Olmo et al. 2001), stilbene glycosides (Son et al. 2007) and resveratrol against a fish parasite *Philasterides dicentrarchi* (Leiro et al. 2004a). Although resveratrol has been shown to be active against bacteria (Chan 2002), fungi (Jung et al. 2005) and viruses (Docherty et al. 1999), there have been no studies to date of its antiparasitic effects on *Leishmania*.

Our results indicate that resveratrol possesses antileishmanial activity when tested in vitro on *L. major* promastigotes. In our system, the IC50 of resveratrol was estimated to be 45 μg/ml, and at 50 μg/ml resveratrol was able to eliminate more than 50% of promastigotes within 48 h. Microscopic examination indicated that the effect was leishmanicidal rather than leishmanistatic, as promastigote morphology indicated cells were dead, rather than their growth was slowed down. More importantly, resveratrol exhibited an antiamastigote activity in the macrophage assay. At 20, 40 and 45 μg/ml, the compound significantly decreased the percentage of infected macrophages within 48 h. The ambiguity (at 20 μg/ml) between activity against intracellular and extracellular forms was previously reported for *Leishmania donovani*, when a group of polyphenols showed good activity against amastigotes but not promastigotes (Kiderlen et al. 2001); however, in our study resveratrol was effective against both forms of *L. major*, albeit at different concentrations. Resveratrol was well-tolerated and non-toxic in rats at doses 1,000 times higher than a normal daily intake (Juan et al. 2002), although renal toxicity was reported after administration of very high doses (1,000 and 3,000 mg/kg body weight per day; Crowell et al. 2004). Therefore, doses higher than reported in this paper may be used, particularly in topical applications against cutaneous leishmaniasis, although it is unknown if high intracellular concentrations of resveratrol can be easily achieved. Resveratrol's suitability for topical application has been demonstrated in treatment of dermatophytes (Chan 2002), at doses of 25–50 μg/ml to treat fungal infections. At these concentrations, resveratrol is expected to be harmless to keratinocytes (Redondo et al. 2000) and fibroblasts (Babich et al. 2000), i.e. host cells mostly affected by treatment of skin infections.

The mechanisms by which resveratrol exerts its direct action on pathogenic microorganisms are still unclear. Leiro et al. (2004b) postulated that against microsporidian *Encephalitozoon cuniculi*, resveratrol either interfered with the polyamine metabolism pathways, exerted antimitotic activity through the inhibition of tubulin polymerisation leading to the arrest of the proliferative phase of development, or alternatively affected spore germination by blocking calcium channels (Dobrydneva et al. 1999). Interestingly in our study, resveratrol was effective at lower concentrations against intracellular amastigotes. Low doses of resveratrol have been shown to shift the cytokine balance towards Th1 type in mice (Feng et al. 2002) and significantly increase the frequency of IFN-γ-producing $CD4^+$ T cells in vitro (Falchetti et al. 2001). Th1-type $CD4^+$ responses and type 1 cytokines such as IFN-γ are essential for acquired immunity to *Leishmania* and considered key factors in immunotherapy and vaccine development (Sacks and Noben-Trauth 2002). Therefore, it is possible that in addition to its direct leishmanicidal effect, resveratrol might also act as an immunomodulator skewing the immune response towards the protective Th1-type. Nonetheless, the literature on the subject is contradictory. In in vitro studies, resveratrol inhibited the induction of cell-mediated cytotoxic responses and production of T cell-secreted cytokines such as IFN-γ (Gao et al. 2001), but in vivo, resveratrol did not affect production of IFN-γ or IL-12 by T cells and macrophages, respectively (Gao et al. 2003).

Most of the hydroxylated resveratrol derivatives tested in this study were shown to have higher antioxidant as well as pro-apoptotic and antiproliferative activity against cancer cells than resveratrol (Murias et al. 2005). All compounds showed antileishmanial activity in vitro, and we chose the most active compounds to further assess their antiparasitic potency and cytotoxicity. At lower concentrations, these compounds exhibited leishmanicidal action but were also toxic to fibroblasts. Compound 16 (3,4,4′,5′-tetrahydroxy-*trans*-stilbene) was the most potent, which might be attributed to presence of two catechol groups, a configuration that shows excellent antioxidant activity if present in a

stilbene structure, possibly also responsible for generation of antioxidant-derived prooxidants (Murias et al. 2005). Such compounds are involved in generation of reactive oxygen species responsible for cytotoxic activity of resveratrol analogues possessing catechol structure. Compound 16 was moderately toxic to macrophages but had a profound effect on the viability of mouse Sp2 myeloid cells. Well prepared for oxidative burst and rich in antioxidative enzymes, macrophages are likely to be less sensitive to reactive oxygen species (ROS) generated during oxidation of catechol groups on compound 16. Paradoxically, macrophage residual amastigotes may be protected against ROS by antioxidant enzymes present in macrophages. However, the lack of activity in the amastigote macrophage assay might also be due to either rapid degradation within macrophages or exclusion by permeability constraints. Additionally, instability of higher hydroxylated analogues in culture medium cannot be ruled out. Therefore, resveratrol seems to possess the "ideal" structure and shows good activity in the macrophage assay.

In recent times, resveratrol and its more potent derivatives showed great promise in treating several leading causes of morbidity and mortality in the developed world such as cancer or heart disease. It is clear that resveratrol possesses therapeutic value in treatment of variety of infectious diseases. Given the need for new drugs for leishmaniasis and in the view of data presented in this paper, resveratrol may be considered for antileishmanial therapy development. In vivo animal trials will be required to confirm this possibility and to fully assess its suitability. Conversely, resveratrol's hydroxylated analogues, although having more potent antioxidant or anticancer activities, need to be further optimised to be suitable as therapeutics against leishmaniasis.

Acknowledgements The authors would like to thank Drs Emanuela Handman and Katherine Kedzierska for critical review of the manuscript. This work was supported by the National Health and Medical Research Council grant (to LK) and NHMRC/ARC Network for Parasitology.

References

Babich H, Reisbaum AG, Zuckerbraun HL (2000) In vitro response of human gingival epithelial S-G cells to resveratrol. Toxicol Lett 114:143–153

Baur JA, Sinclair DA (2006) Therapeutic potential of resveratrol: the in vivo evidence. Nat Rev Drug Discov 5:493–506

Chan MM (2002) Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. Biochem Pharmacol 63: 99–104

Croft SL, Engel J (2006) Miltefosine—discovery of the antileishmanial activity of phospholipid derivatives. Trans R Soc Trop Med Hyg

Crowell JA, Korytko PJ, Morrissey RL, Booth TD, Levine BS (2004) Resveratrol-associated renal toxicity. Toxicol Sci 82:614–619

Davis AJ, Kedzierski L (2005) Recent advances in antileishmanial drug development. Curr Opin Investig Drugs 6:163–169

del Olmo E, Armas MG, Lopez-Perez JL, Munoz V, Deharo E, San Feliciano A (2001) Leishmanicidal activity of some stilbenoids and related heterocyclic compounds. Bioorg Med Chem Lett 11:2123–2126

Desjeux P (2004) Leishmaniasis: current situation and new perspectives. Comp Immunol Microbiol Infect Dis 27:305–318

Dobrydneva Y, Williams RL, Blackmore PF (1999) *trans*-Resveratrol inhibits calcium influx in thrombin-stimulated human platelets. Br J Pharmacol 128:149–157

Docherty JJ, Fu MM, Stiffler BS, Limperos RJ, Pokabla CM, DeLucia AL (1999) Resveratrol inhibition of herpes simplex virus replication. Antivir Res 43:145–155

Eibl H, Unger C (1990) Hexadecylphosphocholine: a new and selective antitumor drug. Cancer Treat Rev 17:233–242

Falchetti R, Fuggetta MP, Lanzilli G, Tricarico M, Ravagnan G (2001) Effects of resveratrol on human immune cell function. Life Sci 70:81–96

Feng YH, Zhou WL, Wu QL, Li XY, Zhao WM, Zou JP (2002) Low dose of resveratrol enhanced immune response of mice. Acta Pharmacol Sin 23:893–897

Gao X, Xu YX, Janakiraman N, Chapman RA, Gautam SC (2001) Immunomodulatory activity of resveratrol: suppression of lymphocyte proliferation, development of cell-mediated cytotoxicity, and cytokine production. Biochem Pharmacol 62:1299–1308

Gao X, Deeb D, Media J, Divine G, Jiang H, Chapman RA, Gautam SC (2003) Immunomodulatory activity of resveratrol: discrepant in vitro and in vivo immunological effects. Biochem Pharmacol 66:2427–2435

Handman E (1999) Cell biology of *Leishmania*. Adv Parasitol 44:1–39

Jha TK, Sundar S, Thakur CP, Bachmann P, Karbwang J, Fischer C, Voss A, Berman J (1999) Miltefosine, an oral agent, for the treatment of Indian visceral leishmaniasis. N Engl J Med 341:1795–1800

Juan ME, Vinardell MP, Planas JM (2002) The daily oral administration of high doses of *trans*-resveratrol to rats for 28 days is not harmful. J Nutr 132:257–260

Jung HJ, Hwang IA, Sung WS, Kang H, Kang BS, Seu YB, Lee DG (2005) Fungicidal effect of resveratrol on human infectious fungi. Arch Pharm Res 28:557–560

Kedzierski L, Montgomery J, Bullen D, Curtis J, Gardiner E, Jimenez-Ruiz A, Handman E (2004) A leucine-rich repeat motif of *Leishmania* parasite surface antigen 2 binds to macrophages through the complement receptor 3. J Immunol 172:4902–4906

Kelleher M, Moody SF, Mirabile P, Osborn AH, Bacic A, Handman E (1995) Lipophosphoglycan blocks attachment of *Leishmania major* amastigotes to macrophages. Infect Immun 63:43–50

Kiderlen AF, Kayser O, Ferreira D, Kolodziej H (2001) Tannins and related compounds: killing of amastigotes of *Leishmania donovani* and release of nitric oxide and tumour necrosis factor alpha in macrophages in vitro. Z Naturforsch [C] 56:444–454

Kolodziej H, Kiderlen AF (2005) Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitised RAW 264.7 cells. Phytochemistry 66:2056–2071

Leiro J, Arranz JA, Parama A, Alvarez MF, Sanmartin ML (2004a) In vitro effects of the polyphenols resveratrol, mangiferin and (−)-epigallocatechin-3-gallate on the scuticociliate fish pathogen *Philasterides dicentrarchi*. Dis Aquat Org 59:171–174

Leiro J, Cano E, Ubeira FM, Orallo F, Sanmartin ML (2004b) In vitro effects of resveratrol on the viability and infectivity of the microsporidian *Encephalitozoon cuniculi*. Antimicrob Agents Chemother 48:2497–2501

Mickuviene I, Kirveliene V, Juodka B (2004) Experimental survey of non-clonogenic viability assays for adherent cells in vitro. Toxicol In Vitro 18:639–648

Mikus J, Steverding D (2000) A simple colorimetric method to screen drug cytotoxicity against *Leishmania* using the dye Alamar Blue. Parasitol Int 48:265–269

Murias M, Handler N, Erker T, Pleban K, Ecker G, Saiko P, Szekeres T, Jager W (2004) Resveratrol analogues as selective cyclooxygenase-2 inhibitors: synthesis and structure-activity relationship. Bioorg Med Chem 12:5571–5578

Murias M, Jager W, Handler N, Erker T, Horvath Z, Szekeres T, Nohl H, Gille L (2005) Antioxidant, prooxidant and cytotoxic activity of hydroxylated resveratrol analogues: structure-activity relationship. Biochem Pharmacol 69:903–912

Murray HW, Berman JD, Davies CR, Saravia NG (2005) Advances in leishmaniasis. Lancet 366:1561–1577

Redondo P, Jimenez E, Perez A, Garcia-Foncillas J (2000) N-acetylcysteine downregulates vascular endothelial growth factor production by human keratinocytes in vitro. Arch Dermatol Res 292:621–628

Sacks D, Noben-Trauth N (2002) The immunology of susceptibility and resistance to *Leishmania major* in mice. Nat Rev Immunol 2:845–858

Siemann EH, Creasy LL (1992) Concentration of the phytoalexin resveratrol in wine. Am J Enol Vitic 43:49–52

Son IH, Chung IM, Lee SJ, Moon HI (2007) Antiplasmodial activity of novel stilbene derivatives isolated from Parthenocissus tricuspidata from South Korea. Parasitol Res 101:237–241

Tapiero H, Tew KD, Ba GN, Mathe G (2002) Polyphenols: do they play a role in the prevention of human pathologies? Biomed Pharmacother 56:200–207