MINIREVIEW
Violacein: properties and biological activities

Nelson Durán*[1], Giselle Z. Justo‡, Carmen V. Ferreira§, Patrícia S. Melo¶, Livia Cordi*† and Dorival Martins*†

**Laboratório de Quimica Biológica, Instituto de Química, Universidade Estadual de Campinas (UNICAMP), C.P. 6154, CEP 13083-970, Campinas, SP, Brazil, †Núcleo de Ciências Ambientais, Universidade de Mogi das Cruzes, Mogi das Cruzes, SP, Brazil, ‡Disciplina de Biologia Molecular, Departamento de Bioquímica, Universidade Federal de São Paulo (UNIFESP/EPM), Rua 3 de maio 100, 4° andar, CEP 04044-020, São Paulo, SP, Brazil, §Laboratório de Transdução de Sinal, Departamento de Bioquímica, Instituto de Biologia, UNICAMP, C.P. 6109, CEP 13083-970, Campinas, SP, Brazil, and ¶Laboratório de Cultura de Células e Biofármacos, Departamento de Bioquímica, Instituto de Biologia, UNICAMP, C.P. 6109, CEP 13083-970, Campinas, SP, Brazil*

The violet pigment violacein is an indole derivative, isolated mainly from bacteria of the genus *Chromobacterium*, which exhibits important antitumoural, antimicrobial and antiparasitary properties. Furthermore, the formulation of violacein in different polymeric carriers developed so far offers alternative approaches to overcoming physiological barriers and undesirable physicochemical properties *in vivo*, thus improving its efficacy.

Introduction

Natural products have had a historical success as biologically active structures and are candidates for therapy [1–8]. In this context, the purple-coloured pigment violacein [3-(1,2-dihydro-5-(5-hydroxy-1*H*-indol-3-yl)-2-oxo-3*H*-pyrrol-3-ilydene)-1,3-dihydro-2*H*-indol-2-one] (Figure 1), produced mainly by bacteria of the genus *Chromobacterium*, has attracted increased interest owing to its important biological activities and pharmacological potential. The biosynthesis of this indole derivative has been extensively studied, and interesting reviews illustrating its production and industrial perspectives, as well as the biological interest in violacein from *Chromobacterium violaceum*, have been published [9–15].

Description

Violacein (molecular mass 343.3), a purplish-black needle prism, is insoluble in water, slightly soluble in ethanol, moderately soluble in dioxane and acetone, and soluble in DMSO, methanol and ethyl acetate. Its melting point is $>290\,^{\circ}C$ (decomposition). The UV–vis (UV and visible absorption) spectrum exhibits maximum absorbances at 258, 372 and 575 nm ($\varepsilon_{575} = 2.97 \pm 0.09 \times 10^{-2}$ ml/μg per cm) in ethanol [16]. The fluorescence emission spectrum at an excitation wavelength of 575 nm shows an emission band at 675 nm [1.5 μg/ml (4.4 μM) in ethanol]. The ^{1}H-NMR, ^{13}C-NMR and IR spectra have been previously discussed [11,16,17].

Biosynthesis

In general, *C. violaceum* is the most studied bacterium in the violacein-production field. However, this pigment is also produced by other bacteria, such as the psychrotrophic bacterium RT102 [18], *Janthinobacterium lividum* (formely known as *Chromobacterium lividum*) [17–21] *and Alteromonas luteoviolacea* [20], but the yield and conditions of production are very variable. The Amazonian strain of *C. violaceum* (from Rio Negro, Brazil) produced violacein at 28 °C at a rate of 4.0 nmol/h per ml [20], as compared with 0.47 nmol/h per ml for the A.T.C.C. 553 strain [22–24]. The 7.5 g/litre dry cell mass and 0.17 g/litre crude violacein yields obtained initially were increased to 21 and 0.43 g/litre respectively [24,25]. Tan et al. [26] reported an apparent high yield of violacein production from the marine-sediment bacterium *Pseudoalteromonas* sp. (DSM 13623 strain) of 25.7 mg of crude violacein/g of cell mass. In contrast, 1.1 g of crude

Key words: antimicrobial, antiparasitary, antitumoral, *Chromobacterium*, indole derivative, violacein.

Abbreviations used: 5-FU, 5-fluorouracil; CD45, receptor-like transmembrane tyrosine phosphatase; EAT, Ehrlich ascites tumour; GI_{50}, concentration inhibiting net cell growth by 50 %; HSV, herpes simplex virus; IC_{50}, the concentration inhibiting cell growth by 50 %; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide; NF-κB, nuclear factor-κB; NRU, Neutral Red uptake; p38 MAPK, p38 mitogen-activated protein kinase; PCL, poly-ε-caprolactone (poly-6-hexanolactone); PLGA, poly(lactic-co-glycollic acid); PTP, protein-tyrosine (-specific) phosphatase; Rb, retinoblastoma protein; RENCA, murine renal carcinoma; SRB, sulforhodamine B; TNF-α, tumour necrosis factor-α; TNFR1, TNF receptor 1; TRAP, tartrate-resistant acid phosphatase; UV–vis, UV and visible.

[1] To whom correspondence should be sent (email duran@iqm.unicamp.br).

© 2007 Portland Press Ltd

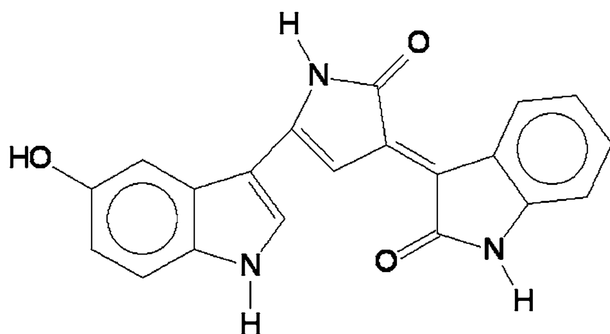

Figure 1 Chemical structure of violacein

violacein/litre from a broth in a 30-litre bioreactor process was recently obtained by our group when the conditions optimized previously by Mendes et al. [24,25] were used.

In addition to violacein purification [27,28], studies were carried out in order to understand its photophysical and phototherapeutic properties [29,30].

Toxicity

Recently, different biochemical parameters were assessed in order to study the interaction between living yeast cells and the microenvironment in the presence of violacein [31]. A dose-dependent oxidation of pyridine nucleotides, a small increase in oxygen uptake and an alteration in the respiratory chain, as evaluated by the oxidation–reduction of cytochrome *c*, was observed. Cytotoxicity studies of violacein in fibroblast V79 cells using different endpoints, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] reduction, nucleic acid content and NRU (Neutral Red uptake), showed cytotoxic activity, with IC_{50} (the concentration inhibiting cell growth by 50%) values in the range 1.7–4.1 μg/ml (5–12 μM) [32–33]. The cytotoxicity of violacein in V79 fibroblasts occurred through apoptosis and not necrosis [33]. The cytotoxicity of violacein was also evaluated in normal human lymphocytes stimulated *in vitro* with phytohaemagglutinin [34]. The MTT assay showed a decrease in lymphocyte proliferation, with an IC_{50} of > 3.4 μg/ml (10 μM), a finding in agreement with the result obtained in the protein phosphatase assay (see below). Using the total-protein-content assay, an IC_{50} of 1 μg/ml (3 μM) was found, reflecting alterations in total protein synthesis [34]. It is important to note that a reduced cytotoxic effect of violacein on normal lymphocytes relative to leukaemia cells [$IC_{50} < 0.34$ μg/ml (< 1 μM)] [35–37] was found, which suggest that violacein is a promising agent in leukaemia therapy. However, in certain cell cultures, at > 0.51 μg/ml (>1.5 μM) concentration, violacein was positive for DNA damage in FRhK-4 (embryonic rhesus-monkey kidney) cells and for both DNA damage and micronuclei in Vero cells (a lineage isolated from African-green-monkey kidney epithelial cells), in a concentration–response relationship [38]. These findings contribute to a comprehensive evaluation of the pharmacological potential of violacein.

Antitumoural activity

As discussed in the previous section, the effect of a compound on a cell line can be evaluated by several assays based on different endpoints that usually describe the percentage growth. The measured effect of the compound is currently expressed as IC_{50} and GI_{50} (the concentration inhibiting net cell growth by 50%). These parameters can be calculated from dose–response curves for the cell lines. IC_{50} is the concentration of test drug where:

$$100 \times T/C = 50\%$$

(T and C are defined below), and GI_{50} is the concentration of the test drug where:

$$100 \times (T - T_0)/(C - T_0) = 50\%$$

thus representing IC_{50} corrected for baseline measured endpoint (T_0), e.g. total cellular protein content or formazan production. The antitumoural activity of violacein had previously been assayed in a panel of tumour-cell lines obtained from the National Cancer Institute (NCI), Bethesda, MD, U.S.A., using the SRB (sulforhodamine B) assay, which involves the determination of the total cellular protein content by absorbance measurements of SRB-derived colour at the time of test drug addition (T_0), and at the end of the experiment in the test drug wells (T) and in the control wells (C). The best results were obtained in MOLT-4 leukaemia, NCI-H460 non-small-cell lung cancer and KM12 colon-cancer cell lines, with GI_{50} values in the range 0.01–0.02 μg/ml (0.03–0.06 μM), which indicates an intermediate potency for violacein [33]. On the basis of these results, the mechanism of action of violacein in different cell lines is currently being investigated [36,37,39,40]. The pigment was also tested in the human uveal melanoma cell lines 92.1 and OCM-1, and the IC_{50} and GI_{50} values were in the range 0.58–1.27 μg/ml (1.69–3.69 μM) [41].

Morphological changes characteristic of apoptosis were induced by violacein in human myeloid leukaemia cells (HL60 cell line) at an IC_{50} of 0.27 μg/ml (0.8 μM) [36,37].

In order to better investigate the molecular mechanisms involved in violacein's cytotoxicity towards leukaemia cells, we have recently demonstrated that violacein-induced apoptosis of HL60 cells occurred through the activation of caspase 8, transcription of NF-κB (nuclear factor-κB) target genes and p38 MAPK (p38 mitogen-activated protein

© 2007 Portland Press Ltd

kinase) activation. These events resemble the activation of TNF-α (tumour necrosis factor-α) signal transduction in these cells. This hypothesis was confirmed by subsequent studies showing that infliximab, an antibody that antagonizes TNF-α-induced signalling, abolished the biological activity of violacein. Importantly, violacein directly and specifically activated TNFR1 (TNF receptor 1) signalling in HL60 cells, since TNF cascade activation was not observed in other leukaemia cell lines (U937 and K562) and normal human blood cells (lymphocytes and monocytes). Hence, violacein represents the first member of a novel class of cytotoxic drugs mediating apoptosis by activation of TNFR1 [36].

Violacein cytotoxicity was also evaluated in human colorectal-cancer cell lines such as HT29 [$IC_{50} > 3.4$ μg/ml (>10 μM), Caco-2 [$IC_{50} = 0.68$ μg/ml (2 μM)], SW480 [$IC_{50} = 0.68$ μg/ml (2 μM)] and DLD1 [$IC_{50} = 0.51$ μg/ml (1.5 μM)] [39,40]. De Carvalho et al. [39] have shown that violacein-induced oxidative stress is a key mediator of cellular apoptosis. Unlike in Caco-2 cells, violacein was incapable of increasing the levels of reactive oxygen species in HT29 cells, which suggests the existence of violacein cell-type-specific mechanisms [39]. Studies were also conducted in HCT116 colon-cancer cells ($IC_{50} = 0.51$ μg/ml (1.5 μM)], which are microsatellite-unstable and resistant to 5-FU (5-fluorouracil) chemotherapy [40]. Violacein caused cell-cycle block at G_1, up-regulated p53, p27 and p21 levels and decreased the expression of cyclin D1. In addition, violacein leads to dephosphorylation of the Rb (retinoblastoma protein) and activation of caspases. Furthermore, the study also provided evidence that violacein acts through the inhibition of Akt (protein kinase B), with subsequent activation of the apoptotic pathway and down-regulation of the NF-κB signalling. This leads to restoration of chemosensitivity to 5-FU in resistant colorectal cancer cells [40].

The role of caspases in the activation of the execution phase of apoptosis induced by violacein was also demonstrated with the murine renal carcinoma [RENCA; $IC_{50} = 0.2$ μg/ml (0.6 μM)] and with Ehrlich tumour [$IC_{50} = 1.7$ μg/ml (5.0 μM)] [42] cells. Data suggest that RENCA cell death induced by violacein is mediated by caspase proteases and that a mitochondrially dependent apoptotic pathway may be involved in violacein-mediated programmed cell death [42]. In addition to the ability of violacein to mediate direct antiproliferative effects against EAT (Ehrlich ascites tumour) cells *in vitro*, its potential was also evaluated *in vivo* [42]. The *in vivo* studies using the intraperitoneal inoculation of EAT cells showed that treatment of mice with 0.1 μg/kg violacein, administered intraperitoneally for 12 consecutive days, significantly increased the lifespan of tumour-bearing mice [42]. This finding constitutes the first evidence of the potential antitumour activity of violacein reported *in vivo*.

The cytotoxicity of violacein in several cell lines is summarized in Table 1.

Table 1 *In vitro* testing results of violacein in several cell lines

Cell line	IC_{50} or GI_{50}* [μg/ml (μM)]	Reference(s)
V79	1.7–4.1 (5.0–12)	[32,33]
Hum. lymph.[a]	>3.4 (>10)	[34]
HL60	0.27 (0.8)	[35–37]
MOLT-4	0.01 (0.03)*	[33], ND[b]
NCI-H460	0.01 (0.03)*	[33], ND[b]
KM12	0.02 (0.06)*	[33], ND[b]
92.1	0.95 (2.78)	[41]
OCM-1	1.27 (3.69)	[41]
HT29	>3.4 (>10)	[39]
Caco-2	0.68 (2.0)	[39,40]
SW480	0.68 (2.0)	[40]
DLD1	0.51 (1.5)	[40]
HCT116	0.51 (1.5)	[40]
RENCA	0.2 (0.6)	[42]
EAT	1.7 (5.0)	[42]
FRhK-4	1.08 (3.14)	[60]
VERO	1.02 (2.96)	[60]
MA104	1.22 (3.55)	[60]
Hep2	1.18 (3.42)	[60]
EOMA	0.17 (0.5)	[42]

[a]Abbreviation: Hum. lymph., human lymphocytes.
[b]Abbreviation: ND, N. Durán, unpublished work.

Enzyme modulation

As mentioned in the previous section, many of the violacein effects are related to alterations in enzyme activities. For instance, violacein presents differential effects on phosphatases isolated from blood serum and human lymphocytes [34]. Total protein phosphatase activity from human lymphocytes was activated after pre-incubation with violacein. Since the active site is highly conserved among the PTPs (protein-tyrosine-specific phosphatases), the effect of violacein on the isolated receptor-like transmembrane tyrosine phosphatase CD45 and intracellular tyrosine phosphatases was evaluated. Under the same conditions, inhibition was achieved for intracellular PTP activity, whereas no alteration was observed on isolated CD45 [34]. In addition, a dose- and time-dependent effect on TRAP (tartrate-resistant acid phosphatase) activity was observed, with 50% inhibition after incubation with 1.7 μg/ml (5 μM) violacein. Since PTP activity is essential for osteoclast function and measurement of the level of bone-specific alkaline phosphatase can be used as an index of bone formation and overall bone turnover, it is plausible to suggest that TRAP could be a molecular target for violacein to be explored in future experiments [34].

Antibacterial, antimycobacterial and antimycotic activities

Singh reported in 1942 [43] the interesting observation that, when the crude extract of violacein was added to bacterial suspensions, these micro-organisms were not ingested by

© 2007 Portland Press Ltd

terrestrial amoebas. There was a marked inhibitory action *in vitro* on the growth of Gram-positive bacteria, with the exception of *Clostridium welchii*, which was moderately resistant, and there were slight inhibitions of the growth of Gram-negative bacteria, except *Neisseria meningitidis*, which was very susceptible. Antimicrobial tests were then conducted in 51 bacteria strains, comprising 21 species, and they showed that violacein possessed an outstanding inhibitory effect on the growth of Gram-positive bacteria and only a small effect on Gram-negative ones [9,44]. Subsequent studies by Durán et al. [10,45] on the antibacterial activity of purified violacein showed that it was efficient against the two groups, with major effects on Gram-positive bacteria. Violacein isolated from *J. lividum* exhibited antimicrobial activity against 21 bacterial strains, encompassing 18 species. The pigment was ineffective on five Gram-negative strains (four species) and one strain of *Bacillus* (*B. subtilis natto*). The growth of *Bacillus* (11 strains, eight species) and *Micrococcus* (two strains, two species) were inhibited with the addition of 2 μg/ml (5.8 μM) violacein. The antimicrobial activity of the pigment was improved by increasing the violacein concentration from 2 (5.8 μM) to 20 μg/ml (58 μM) [46].

The antibacterial action of a mixture of violacein and deoxyviolacein isolated from the psychrotrophic bacterium strain RT102 was demonstrated against Gram-positive *B. lichenniformis*, *B. subtilis*, *B. megaterium*, *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa*, at a high concentration of the pigment. Above 15 μg/ml (43.7 μM), violacein caused not only growth inhibition, but also cell death [18]. However, violacein did not inhibit the growth of Gram-negative bacteria such as *Flavobacterium balustinum*, *Serratia marcences* and *Escherichia coli* or the growth of yeasts such as the soil-living yeast *Trichosporon cutaneum* [18,47]. In addition, violacein acts against phytopathogenic fungi such as *Rosellinia necatrix*, which causes white root rot of mulberry (*Morus* sp.), which suggests its application as a fungicide [19].

Importantly, violacein exhibited *in vitro* antimycobacterial activity against *Mycobacterium tuberculosis* (H37Ra strain). Its effects were comparable with those described in the literature for pyrazinamide, a chemotherapeutic used in the treatment of tuberculosis [48,49].

Antiprotozoal and antiparasitary activities

Purified violacein exhibited high toxicity towards cultures of ciliated protozoa such as *Spumella* sp. and *Ochromonas* sp. [50,51].

The trypanocidal activity of violacein has been described by several authors [52–56]. Violacein was active against the Chilean (Tulahuen) strain and the Brazilian Y strain of *Trypanosoma cruzi*; however, the latter was shown to be three times more resistant than the Tulahuen strain [32,54].

In addition to the biological properties already described, the antileishmanial activity of violacein was tested against promastigote forms of *Leishmania amazonensis* (MHOM/BR/77/LTB0016) [57]. The results showed an effect that was comparable with that of pentamidine, the second drug of choice in the treatment of leishmaniasis. Recently, the anti-plasmodial (antimalarial) activity of violacein was demonstrated *in vitro* and *in vivo* [58]. The *in vivo* efficacy of violacein was investigated in the *Plasmodium chabaudi chabaudi* AS mouse model, and the extension of the anti-plasmodial activity was evaluated daily during the course of infection. In addition, non-infected mice receiving these same doses of violacein did not present any significant reduction in haematocrits. *In vitro* assays against *Plasmodium falciparum* showed that violacein is 300 times more effective than quinine, a chemotherapeutic with known antimalarial activity [58].

Antiviral activity

Violacein (with 10% of deoxyviolacein) was reported to show antiviral activity against HSV (herpes simplex virus) and poliovirus after infection of HeLa cells [59]. At concentrations of about 0.25 μg/ml (0.73 μM), violacein inhibited HSV 62%, and, at 0.063 μg/ml (0.18 μM), it inhibited 56% of poliovirus-infected HeLa cells. These results indicate a modest antiviral activity for the compound.

Andrighetti-Frohner et al. [60] also studied the antiviral and cytotoxic activities of violacein using three different methods. The IC_{50} values were about 1–1.2 μg/ml (2.2–2.7 μM) for FRhK-4, Vero, MA104 and HEp-2 cells. Violacein, at concentrations that did not inhibit cell growth, showed no cytopathic or antiviral activities against HSV-1 (29-R/acyclovir-resistant strain), hepatitis A virus (HN175 and HAF-203 strains) and adenovirus type 5. However, weak inhibition of viral replication of HSV-1 (KOS and ATCC/VR-733 strains), poliovirus type 2 and simian rotavirus SA11 strains was observed when measured using the MTT assay. These results differ from those previously published by May et al. [59], probably because they used different protocols or because the violacein purity differed.

Violacein derivatives

Violacein shows a very low solubility in water, a characteristic that makes some experimental procedures non-feasible, for instance, *in vivo* assays. Therefore, one strategy that is being explored is to synthesize violacein derivatives with glycosidic groups [61,62] or its biotransformation by oxidative enzymes [63,64]. Attempts to increase the solubility and

© 2007 Portland Press Ltd

the biological activity using violacein inclusion complexes (complexes in which one component, the host, forms a cavity) with β-cyclodextrin have also been reported [55,65–69]. In this respect, the ability of violacein to prevent gastric ulceration was potentiated by β-cyclodextrin complexation in different molar ratios [67,68]. Gastroprotective properties similar to, or higher than, that of free violacein were observed for the 1:2 inclusion complex, in different model systems of gastric ulcer in mice, whereas the 1:1 complex was less active than violacein. In addition to their possible role in stimulating the mucosal defensive mechanisms, these studies have also suggested that protection against peroxidative damage may be involved [68]. In agreement with this observation, violacein and its 1:2 inclusion complex were shown to protect isolated rat hepatocytes against peroxidative damage in a dose-dependent way [68,69]. Using a different experimental approach, Sousa et al. [70] demonstrated that violacein prevented the strand-breaking action of Methylene Blue on plasmid DNA. Additionally, a possible role for violacein as an intracellular antioxidant able to protect *C. violaceum* from the oxidative stress has been investigated [71]. These results were reinforced by the antioxidant property of violacein recently reported [72].

The use of biocompatible and biodegradable polymers in micro- and nano-particle formulation contributes to increase the therapeutic efficacy and to reduce the side effects of drugs. Round shape and smooth surface microparticles of PCL [poly-ε-caprolactone (poly-6-hexanolactone)] with diameters of 5–10 μm [violacein concentration 0.25 μg/ml (0.73 μM)] were obtained by the solvent-evaporation technique [73]. Cytotoxicity studies performed on HL60 cells have shown that the incorporation of violacein into PCL microspheres strongly enhanced its cytotoxic activity as compared with free violacein [73]. The cytotoxicity of violacein encapsulated in PLGA [poly(lactic-co-glycollic acid)] microspheres in different tumour cell lines was studied. HL60 cell viability was estimated by the MTT reduction assay, and a correlation between the cytotoxicity and the time of exposure was observed, suggesting that violacein was delivered in a time-dependent manner. However, the formulation of violacein in PLGA microspheres reduced its cytotoxic effect. The same behaviour was observed with RENCA and murine EOMA (haemangioendothelioma) cells [42].

Nanoparticles of PLGA–Pluronic® and of PLGA–Pluronic®–poly(vinyl alcohol) containing violacein were prepared by the nanoprecipitation method (liquid/liquid phase separation) and analysis of the release and the biodegradation processes were carried out [73] (note: Pluronic® is a tradename for a type of block co-polymer based on ethylene oxide and propylene oxide, manufactured by BASF). Nanoparticles containing violacein showed a lower cytotoxicity when they were evaluated by the MTT reduction assay rather than by the phosphatase activity and the Trypan Blue exclusion methods. In addition, it was also shown that the studied formulation was not cytotoxic to cultured liver cells [73]. In addition to the antitumoral activity of violacein co-formulations, the antimalarial properties of violacein incorporated in liposomes, micelles of block co-polymers and in chitosan nano- and micro-structured systems have been described [58].

The recent interest in gold nanoparticles as transfection vectors, DNA-binding agents, protein inhibitors and spectroscopic markers demonstrates the versatility of these systems in biological applications. Monolayers of mono-, multi- and per-thiolated cyclodextrin derivatives with different lengths between the cavity and the thiol terminus adsorbed on to gold films have been prepared by numerous researchers. Recently, this approach was applied to the synthesis of nanoparticles modified with mono-(6-deoxy-6-[(mercaptohexamethylene)thiol])-β-cyclodextrin, a derivative with a medium spacer length in which only one secondary hydroxy group has been replaced by hexane-1,6-dithiol [74]. This research also involved the use of molecular-recognition properties from the novel cyclodextrin–alkylthiol derivatives capping gold nanoparticles to complex the antitumoral violacein. In addition, its cytotoxic properties towards HL60 and V79 cells were compared with those of free violacein. The average particle diameter was 3.8 nm, and physico-chemical characterization was done by Fourier-transform-IR spectroscopic, UV–vis-spectroscopic and transmission-electron-microscopic methodologies. No cytotoxic effects on V79 and HL60 cells were observed for the gold-complexed nanoparticles (without violacein) at the maximum concentration evaluated [(0.1 μg/ml (0.3 μM)]. However, the gold–violacein complex showed less toxicity towards V79 fibroblasts than did free violacein. In HL60 cells, the free violacein exhibited an IC_{50} of 0.27 μg/ml (0.8 μM), whereas for the gold–violacein complex an IC_{50} of 0.62 μg/ml (1.8 μM) was found. These results indicated that the complexation of violacein with gold nanoparticles maintained the cytotoxic properties of violacein *in vitro*. Furthermore, preliminary results suggest a promising application of this system *in vivo* [74,75].

Conclusion

The results presented here provide a basis for the potential clinical application of violacein and for its development in the context of intelligent formulation design.

Acknowledgments

Support from FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo), CNPq (Conselho Nacional de

© 2007 Portland Press Ltd

Desenvolvimento Científico e Tecnológico), Farmabrasilis, Brazilian Nanocosmetic Network [CNPq/MCT (Ministério da Ciência e Tecnologia)] and Brazilian Nanobiotechnology Network (CNPq/MCT) is acknowledged.

References

1 Cragg, G. M., Newman, D. J. and Snader, K. M. (1997) J. Nat. Prod. **60**, 52–60
2 Nielsen, J. (2002) Curr. Opin. Chem. Biol. **6**, 297–305
3 Clardy, J. and Walsh, C. (2004) Nature **432**, 829–837
4 Tsuda, H., Ohshima, Y., Nomoto, H., Fujita, K.-I., Matsuda, E., Iigo, M., Takasuka, N. and Moore, M. A. (2004) Drug Metab. Pharmacokin. **19**, 245–263
5 Justo, G. Z. and Ferreira, C. V. (2005) Curr. Genom. **6**, 461–466
6 Newman, D. J., Cragg, G. M. and Snader, K. M. (2000) Nat. Prod. Rep. **17**, 215–2134
7 Newman, D. J., Cragg, G. M. and Snader, K. M. (2003) J. Nat. Prod. **66**, 1022–2037
8 Rothenberg, M. L., Carbone, D. P. and Johnson, D. H. (2003) Nature Rev. **3**, 303–309
9 De Moss, R. D. (1967) Antibiotics **2**, 77–81
10 Durán, N. (1990) Ciênc. Hoje **11**, 58–80
11 Asamizu, S., Kato, Y., Igarashi, Y. and Onaka, H. (2007) Tetrahedron Lett. **48**, 2923–2926
12 Sanchez, C., Branba, A. F., Mendez, C. and Salas, J. A. (2006) ChemBioChem **17**, 1231–1240
13 Balivar, C. J. and Walsh, C. T. (2006) Biochemistry **45**, 15444–15457
14 Durán, N. and Menck, C. F. M. (2001) Crit. Rev. Microbiol. **27**, 201–222
15 Dessaux, Y., Elmerich, C. and Faure, D. (2004) Rev. Med. Intern. **25**, 659–662
16 Rettori, D. and Durán, N. (1998) World J. Microbiol. Biotechnol. **14**, 685–688
17 Shirata, A., Tsukamoto, T., Yasui, H., Hata, T., Hayasaka, S., Kojima, A. and Kato, H. (2000) Jap. Agric. Res. Q. **34**, 131–140
18 Nakamura, Y., Asada, C. and Sawada, T. (2003) Biotechnol. Bioprocess Eng. **8**, 37–40
19 Shirata, A., Tsukamoto, T., Yasui, H., Kato, H., Hayasaka, S. and Kojima, A. (1997) Nippon Sanshigaku Zasshi **66**, 377–385
20 Laatsch, H. and Thompsom, R. H. (1984) J. Chem. Soc. Perkin Trans. II, 1331–1339
21 Tobie, W. C. (1935) J. Bacteriol. **29**, 223–227
22 Riveros, R., Haun, M. and Durán, N. (1989) Braz. J. Med. Biol. Res. **22**, 569–577
23 De Moss, R. D. and Happel, M. E. (1959) J. Bacteriol. **77**, 137–141
24 Mendes, A. S., De Carvalho, J. E., Duarte, M. C. T., Durán, N. and Bruns, R. (2001) Biotechnol. Lett. **23**, 1963–1969
25 Mendes, A. S., De Carvalho, J. E., Duarte, M. C. T., Durán, N. and Bruns, R. (2001) Braz. Pat. PIBr 0100199-0
26 Tan, T.-L., Montforts, F.-P. and Meyer, D. (2002) PCT Intern Appl. WO 2002050299 A2
27 Durán, N. and Haun, M. (1997) Braz. Pat. PIBr 9702918
28 Rettori, D. and Durán, N. (1997) Braz. Pat. PIBr 9702986-6
29 Durán, N. and Faljoni-Alário, A. (1980) An. Acad. Brasil. Ciênc. **52**, 297–302
30 Dias, Jr, L. C., Neto, J. D. M., Rettori, D. and Durán, N. (2002) J. Mol. Struct. (Theochem.) **580**, 85–90
31 Pereira, R. S., Durán, N. and Volpe, P. L. O. (2005) Eur. J. Drug Metab. Pharmacokinet. **30**, 225–229
32 Haun, M., Pereira, M. F., Hoffman, M. E., Joyas, A., Campos, V., Filardi, L. D., De Castro, S. L. and Durán, N. (1992) Biol. Res. **25**, 21–25
33 Melo, P. S., Maria, S. S., Vidal, B. C., Haun, M. and Durán, N. (2000) In Vitro Cell Dev. Biol. Anim. **36**, 539–543
34 Bromberg, N., Justo, G. Z., Haun, M., Durán, N. and Ferreira, C. V. (2005) J. Enzyme Inhib. Med. Chem. **20**, 449–454
35 Melo, P. S., Justo, G. Z., Xavier, A., De Azevedo, M. B. M., Durán, N. and Haun, M. (2001) FASEB J. **15**, A172
36 Ferreira, C. V., Bos, C. L., Versteeg, H. H., Justo, G. Z., Durán, N. and Peppelenbosch, M. P. (2004) Blood **104**, 1459–1464
37 Melo, P. S., Justo, G. Z., De Azevedo, M. B. M., Durán, N. and Haun, M. (2003) Toxicology **186**, 217–225
38 Andrighetti-Frohner, C. R., Ktatz, J. M., Antonio, R. V., Creczynski-Pasa, T. B., Barardi, C. R. M. and Simões, C. M. O. (2006) Mutation Res. Gen. Toxicol. Environ. Mutagenesis **603**, 97–103
39 De Carvalho, D., Costa, F. T. M., Durán, N. and Haun, M. (2006) Toxicol. In Vitro **20**, 1514–1521
40 Kodach, L. L., Bos, C. L., Durán, N., Peppelenbosh, M. P., Ferreira, C. V. and Hardwick, J. C. (2006) Carcinogenesis **27**, 508–516
41 Saraiva, V. S., Marshall, J.-C., Cools-Lartigue, J. and Burnier, Jr, M. N. (2004) Melanoma Res. **14**, 421–424
42 Justo, G. Z., Ferreira, C. V., Peppelenbosch, M. P. and Durán, N. (2005) ARBS Annual Rev. Biomed. Sci. **7** (Special Issue), 1
43 Singh, B. N. (1942) Nature **149**, 168–170
44 Lichstein, H. C. and Van de Sand, V. F. (1945) J. Infect. Dis. **76**, 47–51
45 Durán, N., Erazo, S. and Campos, V. (1983) An. Acad. Brasil. Ciênc. **55**, 231–234
46 Naito, S., Shiga, I. and Yamaguchi, N. (1986) Nippon Shokuhin Kogyo Gakkaishi **33**, 759–763
47 Nakamura, Y., Sawada, T., Morita, Y. and Tamiya, E. (2002) Biochem. Eng. J. **12**, 79–86
48 De Souza, A. O., Aily, D. C. G., Sato, D. N. and Durán, N. (1999) Rev. Inst. Adolfo Lutz **58**, 59–62
49 Durán, N. and De Souza, A. O. (2001) Braz. Pat. PIBr 0101346–7
50 Kidder, G. W. and Stuart, C. A. (1939) Physiol. Zool. **12**, 329–341

© 2007 Portland Press Ltd

51 Matz, C., Deines, P., Boenigk, J., Arndt, H., Eberl, L., Kjellberg, S. and Jurgens, K. (2004) Appl. Environ. Microbiol. **70**, 1593–1599

52 Caldas, L. R. (1990) Ciênc. Hoje **11**, 56–57

53 Caldas, L. R., Leitão, A. A. C., Santos, S. M. and Tyrrell, R. M. (1978) Intern. Symp. Curr. Topics Radiol. Photobiol. Acad. Brasil. Ciênc., 121–132

54 Durán, N., Campos, V., Riveros, R., Joyas, A., Pereira, M. F. and Haun, M. (1989) An. Acad. Brasil. Ciênc. **61**, 31–36

55 Durán, N., De Azevedo, M. B. M. and Alderete, J. (1998) Braz. Pat. PIBr 9801307

56 Durán, N. and Haun, M. (1991) Mem. Inst. Oswaldo Cruz **86** (Suppl. I), 29–30

57 Leon, L. L., Miranda, C. C., De Souza, A. O. and Durán, N. (2001) J. Antimicrob. Chemother. **48**, 445–458

58 Costa, F. T. M., Lopes, S. C. P., Nogueira, P. A., Justo, G. Z. and Durán, N. (2006) Braz. Pat. PIBr 0506399–0

59 May, G., Brummer, B. and Ott, H. (1991) Ger. Pat. DE 3935066

60 Andrighetti-Frohner, C. R., Antonio, R. V., Creczynski-Pasa, T. B., Barandi, C. R. M. and Simões, C. M. O. (2003) Mem. Inst. Oswaldo Cruz **98**, 834–848

61 Durán, N., De Conti, R., Sato, L. M., Jardim, L. S. A. and Moraes, S. G. (1997) Braz. Pat. PIBr 9702822

62 Durán, N., Jardim, L. S. A., Moraes, S. G. and De Conti, R. (1998) Rev. Soc. Quim. Chile **43**, 91–96

63 Bromberg, N. and Durán, N. (2001) J. Mol. Catal. B Enzymol. **11**, 463–467

64 Bromberg, N. and Durán, N. (2001) Lett. Appl. Microbiol. **33**, 316–319

65 De Azevedo, M. B. M., Alderete, J., Lino, A. C. S., Loh, W., Faljoni-Alario, A. and Durán, N. (2000) J. Incl. Phenon. Macrocyclic Chem. **37**, 67–74

66 De Azevedo, M. B. M., Alderete, J., Rodriguez, J. A., De Souza, A. O., Rettori, D., Torsoni, M. A., Faljoni-Alario, A., Haun, M. and Durán, N. (2000) J. Incl. Phenon. Macrocyclic Chem. **37**, 93–101

67 De Azevedo, M. B. M., Melo, P. S., Almeida, A. B. A., Souza-Brito, A. R. M., Haun, M. and Durán, N. (2000) Proc. Intern. Symp. Control. Rel. Bioact. Mater. **27**, 508–509

68 Durán, N., Justo, G. Z., Melo, P. S., De Azevedo, M. B. M., Souza-Brito, A. R. M., Almeida, A. B. A. and Haun, M. (2003) Can. J. Physiol. Pharmacol. **81**, 387–396

69 De Azevedo, M. B. M., Justo, G. Z., Rettori, D., Rodriguez, J. A., Haun, M. and Durán, N. (2000) Proc. Intern. Symp. Control. Rel. Bioact. Mater. **27**, 506–507

70 Sousa, A. O., Egito, L. C. M., Medeiros, S. R. B. and Agnez-Lima, L. F. (2005) Genet. Mol. Biol. **28**, 183–185

71 Rettori, D., Rodriguez, R. A. and Durán, N. (1998) Rev. Farm. Bioquím. Univ. São Paulo **34** (Suppl. 1), 169–169

72 Konzen, M., De Marco, D., Cordova, C. A. S., Vieira, T. O., Antonio, R. V. and Creczynski-Pasa, T. B. (2006) Biorg. Med. Chem. **14**, 8307–8313

73 Durán, N. and De Azevedo, M. M. M. (2004) Braz. Pat. PIBr 0100199-0

74 Gimenez, I. F., Anazetti, M. C., Melo, P. S., Haun, M., De Azevedo, M. M. M., Durán, N. and Alves, O. L. (2005) J. Biomed. Nanotechnol. **1**, 352–358

75 Alves, O. L., Gimenez, I. F., Melo, P. S., De Azevedo, M. M. M. and Durán, N. (2005) Braz. Pat. PIBr 0502657-1

Received 16 May 2007/13 July 2007; accepted 20 July 2007
Published on the Internet 15 October 2007, doi:10.1042/BA20070115

© 2007 Portland Press Ltd