Lipid-core nanocapsules increase the oral efficacy of quercetin in cutaneous leishmaniasis

A. J. SOUSA-BATISTA[1], F. S. POLETTO[2], C. I. M. S. PHILIPON[1], S. S. GUTERRES[3], A. R. POHLMANN[2] *and* B. ROSSI-BERGMANN[1]*

[1] *Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Av Carlos Chagas Filho 373, 21941-901 Rio de Janeiro, RJ, Brazil*
[2] *Instituto de Química, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, 91501-970 Porto Alegre, RS, Brazil*
[3] *Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, 90610-000 Porto Alegre, RS, Brazil*

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SUMMARY

New oral treatments are needed for all forms of leishmaniasis. Here, the improved oral efficacy of quercetin (Qc) and its penta-acetylated derivative (PQc) was evaluated in cutaneous leishmaniasis after encapsulation in lipid-core nanocapsules (LNCs) of poly(ε-caprolactone). *Leishmania amazonensis*-infected BALB/c mice were given 51 daily oral doses of free drugs (16 mg kg^{-1}) or LNC-loaded drugs (0·4 mg kg^{-1}). While treatment with free Qc reduced the lesion sizes and parasite loads by 38 and 71%, respectively, LNC-Qc produced 64 and 91% reduction, respectively. The antileishmanial efficacy of PQc was similar but not as potently improved by encapsulation as Qc. None of the treatments increased aspartate aminotransferase, alanine aminotransferase or creatinine serum levels. These findings indicate that when encapsulated in LNC, Qc and, to a lesser extent, PQc can safely produce an enhanced antileishmanial effect even at a 40-fold lower dose, with implications for the development of a new oral drug for cutaneous leishmaniasis.

Key words: leishmania, chemotherapy, LNC, drug delivery, nanoparticle, nanotechnology.

INTRODUCTION

Leishmaniasis is a complex of neglected tropical diseases caused by different species of protozoa of the genus *Leishmania*, transmitted by the bite of phlebotomine sand flies. The clinical forms vary from the morbid and most common cutaneous leishmaniasis (CL) causing 1 000 000 annual new cases worldwide to fatal visceral leishmaniasis (VL) (World Health Organization, 2015).

As there are no vaccines, drugs remain the most important tool for the control of human leishmaniasis (Srivastava *et al*. 2016). In comparison with VL, there are limited treatment options for CL, in which the response to injectable pentavalent antimonials, amphotericin B lipid formulations, pentamidine and oral miltefosine is unpredictable and/or may cause severe adverse effects (Monge-Maillo and López-Vélez, 2015; Sundar and Chakravarty, 2015). Local therapies have found variable degrees of efficacy (Navin *et al*. 1990; López *et al*. 2012; Ben Salah *et al*. 2013; Sosa *et al*. 2013), and so new safe drugs particularly for local or oral use that increase patient compliance are urgently needed for CL.

Natural and synthetic flavonoids, such as chalcones and quercetin (Qc, Fig. 1), have been reported with safe antileishmanial properties (Torres-Santos *et al*. 1999; Sen *et al*. 2005; Boeck *et al*. 2006; Muzitano *et al*. 2009; Sen and Chatterjee, 2011). Qc is the most abundant antioxidant of fruits and vegetables, with a wide range of therapeutic actions including chemoprevention (Gibellini *et al*. 2010), anti-inflammatory (Li *et al*. 2016*a*) and antiallergic activities (Mlcek *et al*. 2016). Qc and its penta-acetylated derivative (PQc, Fig. 1B) were previously demonstrated with *in vitro* activity against *Leishmania* spp. (Muzitano *et al*. 2006; Marín *et al*. 2009). Its antileishmanial effect seems to be related to the inhibition of parasite arginase, an important enzyme in reactive oxygen species detoxification mechanisms (Fonseca-Silva *et al*. 2011). *In vivo*, we and others have shown the oral efficacy of Qc in murine CL caused by *Leishmania amazonensis* (Muzitano *et al*. 2009) and in hamster VL caused by *Leishmania donovani* (Sen *et al*. 2005). Therefore, Qc is a good oral compound prototype for further development.

Since the lipophilic nature of Qc may impair its oral absorption, lipid nanosystems capable of drug protection against gastric digestion while promoting intestinal uptake would be ideal carriers for Qc oral delivery (Li *et al*. 2009; Sun *et al*. 2010; Tran *et al*. 2014). In this sense, lipid-core nanocapsules (LNCs) made of a poly(ε-caprolactone) shell have emerged as a safe carrier system to improve intestinal uptake *in vivo* (Frozza *et al*. 2010; Venturini *et al*. 2011).

* Corresponding author: Instituto de Biofísica Carlos Chagas Filho, Av Carlos Chagas Filho 373, 21·9410901 Rio de Janeiro, RJ, Brazil. E-mail: bartira@biof.ufrj.br

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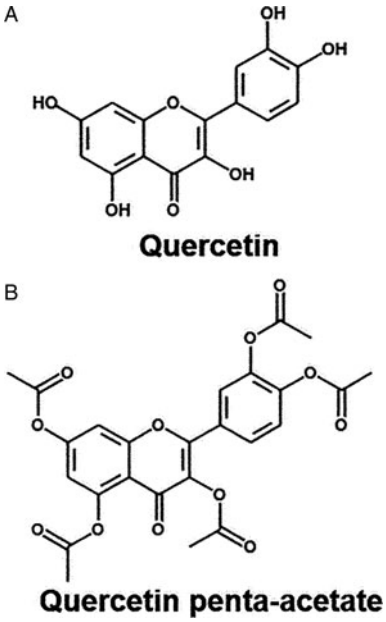

Fig. 1. Chemical structures of (A) Qc and (B) PQc.

Besides their potential to increase oral drug bioavailability (Frozza *et al*. 2010), *in vitro* studies using rhodamine *B*-labelled LNCs showed that they can be effectively internalized and compartmentalized inside the phagocytic vacuoles of macrophages (Poletto *et al*. 2012), the host cells of *Leishmania* parasites. Recently, we described the production and characterization of LNCs loaded with Qc and PQc (LNC-Qc and LNC-PQc, respectively) (Poletto *et al*. 2015). By introducing sorbitan monostearate into the oil core, the drug-loading rate could be increased by 40-fold as compared with conventional nanocapsules.

Thus, both LNC-Qc and LNC-PQc formulations with maximized drug loadings seemed suitable for oral administration and were evaluated here for their capacity to treat CL in mice infected with *L. amazonensis*.

MATERIALS AND METHODS

Chemicals

Qc was supplied by Henrifarma (São Paulo, Brazil). PQc was synthesized from Qc by our group as previously described (Poletto *et al*. 2015). Poly(ε-caprolactone) (PCL; MW = 14 000 g mol) was obtained from Aldrich (Strasbourg, France); sorbitan monostearate (Span 60®) was from Sigma (St. Louis, Missouri, USA); and caprylic/capric triglyceride was acquired from Alpha Química (Porto Alegre, Brazil). Polysorbate 80 was supplied from Gerbras (São Paulo, Brazil). All chemicals and solvents were of analytical or pharmaceutical grade.

LNC-Qc and LNC-PQc preparation

LNC-Qc and LNC-PQc were prepared in aqueous suspensions by interfacial deposition of preformed polymer (Poletto *et al*. 2015). Briefly, 0·400 mg of Qc or 0·500 mg of PQc was added to the organic phase comprising 0·100 g of poly(ε-caprolactone), 0·16 mL of capric/caprylic triglyceride, 0·040 g of sorbitan monostearate and 27 mL of acetone at 40 °C. These organic phases were injected into 53 mL of an aqueous phase containing 80 mg of polysorbate 80 at 40 °C, under moderate stirring for 10 min. Finally, the acetone was eliminated and water partially evaporated to approximately 9 mL under reduced pressure at 40 °C (Rotative evaporator, Buchi, Switzerland). Then, the volume was adjusted in a volumetric flask to 10 mL. This process yielded LNC-Qc and LNC-PQc nanocapsules with unimodal size distributions with volume-weighted diameters of 222 and 217 nm with a polydispersity (expressed as span) lower than 1·8. Liquid chromatography showed compound contents of 40·2 and 50 μg mL^{-1}, respectively.

Mice

BALB/c mice originally purchased from Jackson Laboratory (Bar Harbor, Maine, USA) were bred and maintained in our facilities at Federal University of Rio de Janeiro under controlled temperature, with filtered air, filtered water, autoclaved bedding and pelleted food. Female mice at 8 weeks of age (approximately 20 g of body weight) were used in this study. All experiments were performed in conformity with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, NIH) and were approved by the Committee on the Ethics of Animal Use of the Federal University of Rio de Janeiro under the code CAUAP180.

Treatment efficacy

The efficacy of Qc, PQc and their LNC formulations was evaluated in a murine model of CL. For this purpose, BALB/c mice were infected in the ear with 2×10^6 promastigotes of *L. amazonensis* (MHOM/BR/75/Josefa strain) transfected with green fluorescent protein (*L. amazonensis*-GFP) (Rossi-Bergmann *et al*. 1999). Seven days after infection, animals were treated daily by intragastric gavage with 200 μL of LNC-Qc or LNC-PQc (0·4 mg of drug kg^{-1}) or with the free drugs Qc or PQc (16 mg kg^{-1}) in 200 μL of soybean oil plus 2% ethanol as previously (Muzitano *et al*. 2009) for 51 days. Controls received 200 μL of soybean oil plus 2% ethanol by the oral route. For clinical follow-up, ear thicknesses were periodically measured using a dial caliper. For parasite burden evaluation, the animals were euthanized with isofluorane overdose on day 59 of infection, and the infected ears were surgically removed. The tissues were homogenized in 1 mL of phosphate-buffered saline with a tissue grinder. The parasite loads were quantified

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in the single-cell suspensions by both limiting dilution assay (LDA) (Lima *et al*. 1997) and fluorimetry (435 nm excitation and 538 nm emission – *FLx800, Bio-Tek Instruments, Winooski, Vermont, USA*) (Demicheli *et al*. 2004).

Toxicity studies

Mice were treated as above with the free drugs and their nanoformulations. In the next day after treatment suspension (day 59), the levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine were measured in the individual sera using commercial analytical kits (Doles, Brazil) adapted for microvolumes (da Cunha-Júnior *et al*. 2011). Positive sera were from mice receiving 200 μL of 1% carbon tetrachloride in soybean oil by the intraperitoneal route 3 days before the experiment (Otsuka *et al*. 2002). The normal reference was sera from untreated mice.

Statistical analysis

Data were statistically analysed by Student's *t*-test or one-way analysis of variance with the Tukey's post-test using GraphPad Prism 6 software. Values were considered different when $P < 0{\cdot}05$.

RESULTS AND DISCUSSION

Leishmania amazonensis-infected BALB/c were used here as a sensitive experimental model of CL to test the oral efficacy of LNC-Qc and LNC-PQc. The free drugs were given in therapeutic doses (16 mg kg^{-1}) (Muzitano *et al*. 2009), whereas the LNC formulations were given in doses 40-fold smaller (0·4 mg kg^{-1}), the maximal intragastric dose possible in 200 μL. Qc at 0·4 mg kg^{-1} produces no effect in CL using the same treatment protocol (not shown). Figure 2A shows that from day 40 of infection, all treatments prevented lesion growth. When data are depicted separately as in day 58 (Fig. 2B), the efficacy of PQc appears slightly higher than Qc, reducing lesion sizes by 38 and 47%, respectively, but this difference is not statistically significant ($P > 0{\cdot}05$). This is in agreement with the similar *in vitro* activities of both compounds against *Leishmania* (V.) *peruviana* and *Leishmania* (V.) *braziliensis* promastigotes (Marín *et al*. 2009) and is the first *in vivo* demonstration of PQc efficacy in leishmaniasis. Besides, this finding indicates that introduction of acetyl groups does not affect Qc antileishmanial activity. Treatment with LNC-Qc led to lesions significantly smaller ($P < 0{\cdot}05$) than those obtained with Qc, indicative of a clear increase in drug potency after encapsulation. The same can be said about LNC-PQc and PQc, considering the 40-fold lower dose in the former.

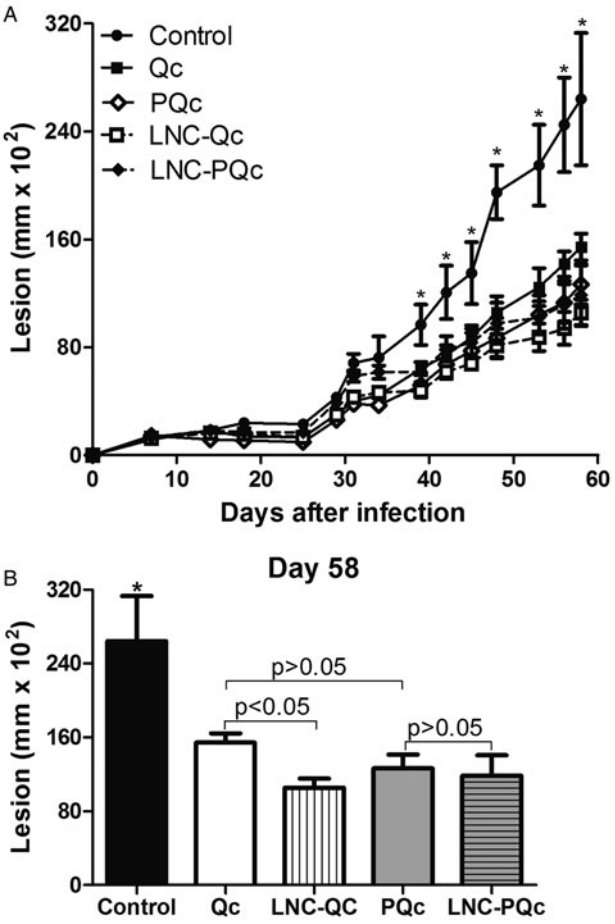

Fig. 2. Clinical cure evaluation. BALB/c mice were infected in the right ear with 2×10^6 promastigotes of *Leishmania amazonensis*-GFP. On days 7–58 of infection, animals were daily treated with 16 mg kg^{-1} of the free drugs Qc or PQc, or 0·4 mg kg^{-1} of the encapsulated drugs LNC-Qc or LNC-PQc by the oral route. The control group received vehicle alone (200 μL^{-1} of soybean oil plus 2% ethanol). (A) Infected and non-infected ear thicknesses were measured in the indicated days with a digital caliper. Lesion sizes were expressed as the difference between them in each time point. (B) The lesion sizes picked in day 58 in (A) were expressed in bars. These results are representative of three different experiments. Means ± S.E.M. ($n = 5$). *$P < 0{\cdot}05$ in relation to all treated groups.

Lesion thickness should not be used as a sole efficacy parameter due to the possibility of inflammation and oedema. For greater accuracy, at the end of the treatment, the parasite loads were estimated by an indirect (fluorimetry) and direct (LDA) assay. The superior effectiveness of LNC-Qc in relation to Qc was confirmed in Fig. 3A and B. When the 40-fold lower drug intake is taken into account, an 80- to 160-fold decrease in parasite loads after drug encapsulation into LNC is assumed using each method. These findings are in line with previous studies showing increased Qc bioavailability after encapsulation in solid lipid nanoparticles (Li *et al*. 2009), self-nanoemulsifying system (Tran *et al*. 2014) or nanosuspension (Sun *et al*. 2010).

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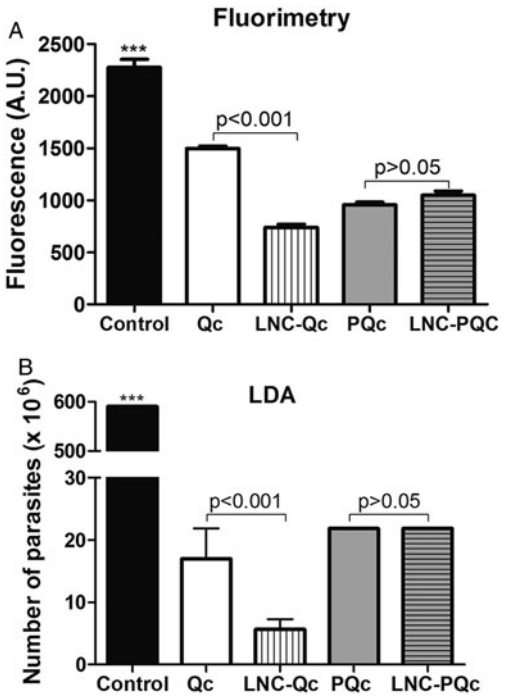

Fig. 3. Parasitological cure evaluation. BALB/c mice were infected and treated as in Fig. 1. After 59 days of infection, the animals were euthanized, and parasite burden was determined by (A) fluorimetry and (B) LDA. Fluorimetric values are expressed as arbitrary units (A.U.) and are corrected for background uninfected ear values (A.U. = 2083). These results are representative of three different experiments. Mean ± S.E.M. ($n = 5$). *$P < 0{\cdot}05$ and **$P < 0{\cdot}001$ in relation to all treated groups.

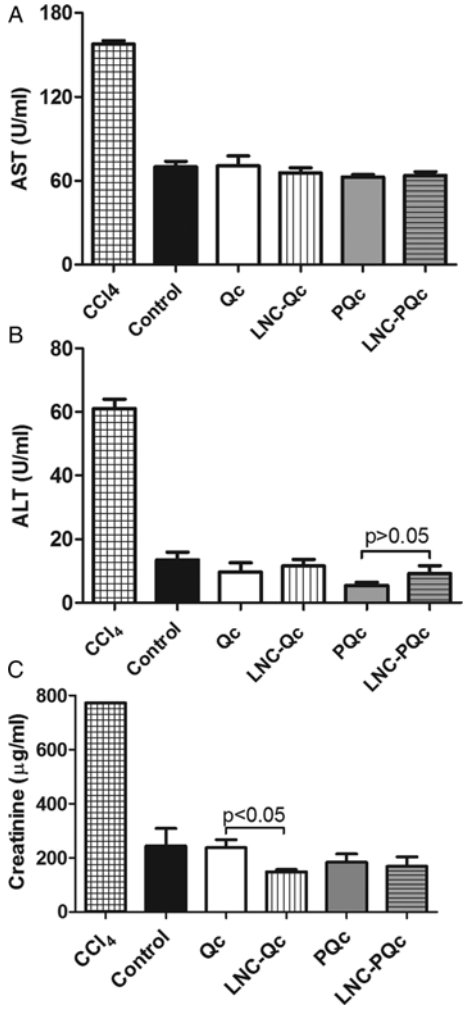

Fig. 4. Chronic toxicity evaluation. BALB/c mice were infected and treated as in Fig. 1. At the end of the treatment, the serum from each mouse was collected to evaluate the cardiac, hepatic, and renal toxicity by measuring the indicated enzyme concentrations. CCl_4 is positive sera obtained from animals pretreated with CCl_4. The control is the reference sera from untreated mice. These results are representative of two different experiments. Mean ± S.E.M. ($n = 5$).

The reason why encapsulation in LNC greatly increases Qc efficacy may be associated with at least one of the steps from oral intake to drug release inside the infected cells. The assumption that the LNC polymeric shell protects Qc against extensive gastric and intestinal degradation and elimination as reported earlier (Graf *et al*. 2006) is supported by the better sustained antioxidant activity exhibited by Qc encapsulated in LNC also containing octyl methoxycinnamate in the lipophilic core (Weiss-Angeli *et al*. 2012). LNCs are orally absorbed intact, as previously demonstrated in the treatment of glioma in mice (Rodrigues *et al*. 2016). Nanoparticle uptake by intestinal *M* cells may also play an important role in oral absorption (Lopes *et al*. 2014). In addition, the polymeric shell may prevent active Qc conjugation with the small-intestine cells (Crespy *et al*. 1999), thus allowing better drug absorption. Finally, LNC may confer structural integrity in blood circulation (Li *et al*. 2016*b*) up to internalization by *Leishmania*-infected macrophages in inflamed lesions. Oral biodistribution studies with free and LNC-encapsulated compounds particularly in the infected tissue would help clarify this issue. Treatment toxicity was assessed by measuring serum AST, ALT and creatinine, whose elevated levels are markers of cardiac, hepatic and kidney damage, respectively. After 51 doses, when treatment toxicity is expected to be maximum, none of those markers were elevated (Fig. 4), indicative of treatment safety. That was further supported by the observation that mouse weight gain was not statistically different amongst all the groups throughout treatment (not shown). These findings are in agreement with other studies demonstrating the safety of nanocapsules containing poly(ε-caprolactone) as a polymeric matrix (Pohlmann *et al*. 2013). The oral safety of blank LNCs as used here has also been demonstrated in an acute and subchronic treatment in rats (Bulcão *et al*. 2013). As to Qc safety, only after a long period (2 years) of daily consumption of high Qc dose (1900 mg kg^{-1} day^{-1}) did Qc show carcinogenic activity (Dunnick and Hailey, 1992): a toxic Qc dose that was far higher (100-fold) than the

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dose used previously (Muzitano *et al*. 2009) and in the present work (16 mg kg^{-1} day^{-1}), and it was considerably decreased when using LNC.

Concluding remarks

We conclude that encapsulation in LNC safely increases the oral efficacy of Qc and, to a lesser extent, its PQc derivative in the murine model of CL. These nanosystems have good oral potential not only in CL but also all conditions where Qc may show therapeutic potential.

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REFERENCES

Ben Salah, A., Ben Messaoud, N., Guedri, E., Zaatour, A., Ben Alaya, N., Bettaieb, J., Gharbi, A., Belhadj Hamida, N., Boukthir, A., Chlif, S., Abdelhamid, K., El Ahmadi, Z., Louzir, H., Mokni, M., Morizot, G., Buffet, P., Smith, P. L., Kopydlowski, K. M., Kreishman-Deitrick, M., Smith, K. S., Nielsen, C. J., Ullman, D. R., Norwood, J. A., Thorne, G. D., McCarthy, W. F., Adams, R. C., Rice, R. M., Tang, D., Berman, J., Ransom, J. *et al*. (2013). Topical paromomycin with or without gentamicin for cutaneous leishmaniasis. *New England Journal of Medicine* **368**, 524–532.

Boeck, P., Bandeira Falcão, C. A., Leal, P. C., Yunes, R. A., Filho, V. C., Torres-Santos, E. C. and Rossi-Bergmann, B. (2006). Synthesis of chalcone analogues with increased antileishmanial activity. *Bioorganic and Medicinal Chemistry* **14**, 1538–1545.

Bulcão, R. P., Freitas, F. A., Venturini, C. G., Dallegrave, E., Durgante, J., Göethel, G., Cerski, C. T. S., Zielinsky, P., Pohlmann, A. R., Guterres, S. S. and Garcia, S. C. (2013). Acute and subchronic toxicity evaluation of poly(e-caprolactone) lipid-core nanocapsules in rats. *Toxicological Sciences* **132**, 162–176.

Crespy, V., Morand, C., Manach, C., Besson, C., Demigne, C. and Remesy, C. (1999). Part of quercetin absorbed in the small intestine is conjugated and further secreted in the intestinal lumen. *American Journal of Physiology* **277**, G120–G126.

da Cunha-Júnior, E. F., Pacienza-Lima, W., Ribeiro, G. A., Netto, C. D., do Canto-Cavalheiro, M. M., da Silva, A. J. M., Costa, P. R. R., Rossi-Bergmann, B. and Torres-Santos, E. C. (2011). Effectiveness of the local or oral delivery of the novel naphthopterocarpanquinone LQB-118 against cutaneous leishmaniasis. *Journal of Antimicrobial Chemotherapy* **66**, 1555–1559.

Demicheli, C., Ochoa, R., da Silva, J. B. B., Falcão, C. A. B., Rossi-Bergmann, B., de Melo, A. L., Sinisterra, R. D. and Frézard, F. (2004). Oral delivery of meglumine antimoniate-beta-cyclodextrin complex for treatment of leishmaniasis. *Antimicrobial Agents and Chemotherapy* **48**, 100–103.

Dunnick, J. and Hailey, J. (1992). Toxicity and carcinogenicity studies of quercetin, a natural component of foods. *Fundamental and Applied Toxicology* **19**, 423–431.

Fonseca-Silva, F., Inacio, J. D. F., Canto-Cavalheiro, M. M. and Almeida-Amaral, E. E. (2011). Reactive oxygen species production and mitochondrial dysfunction contribute to quercetin induced death in *Leishmania amazonensis*. *PLoS ONE* **6**, e14666.

Frozza, R., Bernardi, A., Paese, K., Hoppe, J., da Silva, T., Battastini, A., Pohlmann, A., Guterres, S. and Salbego, C. (2010). Characterization of trans-resveratrol-loaded lipid-core nanocapsules and tissue distribution studies in rats. *Journal of Biomedical Nanotechnology* **6**, 694–703.

Gibellini, L., Pinti, M., Nasi, M., Montagna, J. P., De Biasi, S., Roat, E., Bertoncelli, L., Cooper, E. L. and Cossarizza, A. (2010). Quercetin and cancer chemoprevention. *Evidence-based Complementary and Alternative Medicine* **2011**, 1–15.

Graf, B. A., Ameho, C., Dolnikowski, G. G., Milbury, P. E., Chen, C.-Y. and Blumberg, J. B. (2006). Rat gastrointestinal tissues metabolize quercetin. *Journal of Nutrition* **136**, 39–44.

Li, Y., Yao, J., Han, C., Yang, J., Chaudhry, M. T., Wang, S., Liu, H. and Yin, Y. (2016*a*). Quercetin, inflammation and immunity. *Nutrients* **8**, 1–14.

Li, H., Chen, M., Su, Z., Sun, M. and Ping, Q. (2016*b*). Size-exclusive effect of nanostructured lipid carriers on oral drug delivery. *International Journal of Pharmaceutics* **511**, 524–537.

Li, H., Zhao, X., Ma, Y., Zhai, G., Li, L. and Lou, H. (2009). Enhancement of gastrointestinal absorption of quercetin by solid lipid nanoparticles. *Journal of Controlled Release* **133**, 238–244.

Lima, H. C., Bleyenberg, J. A. and Titus, R. G. (1997). A simple method for quantifying *Leishmania* in tissues of infected animals. *Parasitology Today* **13**, 80–82.

Lopes, M., Abrahim, B., Cabral, L., Rodrigues, C., Seiça, R., de Baptista, F. and Ribeiro, A. (2014). Intestinal absorption of insulin nanoparticles: contribution of M cells. *Nanomedicine* **10**, 1139–1151.

López, L., Robayo, M., Vargas, M. and Vélez, I. D. (2012). Thermotherapy. An alternative for the treatment of American cutaneous leishmaniasis. *Trials* **13**, 1–7.

Marín, C., Boutaleb-Charki, S., Díaz, J. G., Huertas, O., Rosales, M. J., Pérez-Cordon, G., Guitierrez-Sánchez, R. and Sánchez-Moreno, M. (2009). Antileishmaniasis activity of flavonoids from *Consolida oliveriana*. *Journal of Natural Products* **72**, 1069–1074.

Mlcek, J., Jurikova, T., Skrovankova, S. and Sochor, J. (2016). Quercetin and its anti-allergic immune response. *Molecules* **21**, 1–15.

Monge-Maillo, B. and López-Vélez, R. (2015). Miltefosine for visceral and cutaneous leishmaniasis: drug characteristics and evidence-based treatment recommendations. *Clinical Infectious Diseases* **60**, 1398–1404.

Muzitano, M. F., Tinoco, L. W., Guette, C., Kaiser, C. R., Rossi-Bergmann, B. and Costa, S. S. (2006). The antileishmanial activity assessment of unusual flavonoids from *Kalanchoe pinnata*. *Phytochemistry* **67**, 2071–2077.

Muzitano, M. F., Falcão, C. A. B., Cruz, E. A., Bergonzi, M. C., Bilia, A. R., Vincieri, F. F., Rossi-Bergmann, B. and Costa, S. S. (2009). Oral metabolism and efficacy of *Kalanchoe pinnata* flavonoids in a murine model of cutaneous leishmaniasis. *Planta Medica* **75**, 307–311.

Navin, T. R., Arana, B. A., Arana, F. E., de Mérida, A. M., Castillo, A. L. and Pozuelos, J. L. (1990). Placebo controlled clinical trial of meglumine antimonate (glucantime) vs. localized controlled heat in the treatment of cutaneous leishmaniasis in Guatemala. *American Journal of Tropical Medicine and Hygiene* **42**, 43–50.

Otsuka, T., Takagi, H., Horiguchi, N., Toyoda, M., Sato, K., Takayama, H. and Mori, M. (2002). CCl4-induced acute liver injury in mice is inhibited by hepatocyte growth factor overexpression but stimulated by NK2 overexpression. *FEBS Letters* **532**, 391–395.

Pohlmann, A. R., Fonseca, F. N., Paese, K., Detoni, C. B., Coradini, K., Beck, R. C. R. and Guterres, S. S. (2013). Poly (e-caprolactone) microcapsules and nanocapsules in drug delivery. *Expert Opinion on Drug Delivery* **10**, 623–638.

Poletto, F. S., Fiel, L. A., Lopes, M. V., Schaab, G., Gomes, A. M. O., Guterres, S. S., Rossi-Bergmann, B. and Pohlmann, A. R. (2012). Fluorescent-labeled poly(ε-caprolactone) lipid-core nanocapsules: synthesis, physicochemical properties and macrophage uptake. *Journal of Colloid Science and Biotechnology* **1**, 89–98.

Poletto, F. S., De Oliveira, C. P., Wender, H., Regent, D., Donida, B., Teixeira, S. R., Guterres, S. S., Rossi-Bergmann, B. and Pohlmann, A. R. (2015). How sorbitan monostearate can increase drug-loading capacity of lipid-core polymeric nanocapsules. *Journal of Nanoscience and Nanotechnology* **15**, 827–837.

Rodrigues, S. F., Fiel, L. A., Shimada, A. L., Pereira, N. R., Guterres, S. S., Pohlmann, A. R. and Farsky, S. H. (2016). Lipid-core nanocapsules act as a drug shuttle through the blood brain barrier and reduce glioblastoma after intravenous or oral administration. *Journal of Biomedical Nanotechnology* **12**, 986–1000.

Rossi-Bergmann, B., Lenglet, A., Bezerra-Santos, C., Costa-Pinto, D. and Traub-Czeko, Y. (1999). Use of fluorescent *Leishmania* for faster quantification of parasite growth in vitro and in vivo. *Memorias do Instituto Oswaldo Cruz* **94**, 74.

Sen, R. and Chatterjee, M. (2011). Plant derived therapeutics for the treatment of leishmaniasis. *Phytomedicine* **18**, 1056–1069.

Sen, G., Mandal, S., Roy, S. S., Mukhopadhyay, S. and Biswas, T. (2005). Therapeutic use of quercetin in the control of infection and anemia associated with visceral leishmaniasis. *Free Radical Biology and Medicine* **38**, 1257–1264.

Sosa, N., Capitán, Z., Nieto, J., Nieto, M., Calzada, J., Paz, H., Spadafora, C., Kreishman-Deitrick, M., Kopydlowski, K.,

Ullman, D., McCarthy, W. F., Ransom, J., Berman, J., Scott, C. and Grogl, M. (2013). Randomized, double-blinded, phase 2 trial of WR 279,396 (paromomycin and gentamicin) for cutaneous leishmaniasis in Panama. *American Journal of Tropical Medicine and Hygiene* **89**, 557–563.

Srivastava, S., Shankar, P., Mishra, J. and Singh, S. (2016). Possibilities and challenges for developing a successful vaccine for leishmaniasis. *Parasites & Vectors* **9**, 277–292.

Sun, M., Gao, Y., Pei, Y., Guo, C., Li, H., Cao, F., Yu, A. and Zhai, G. (2010). Development of nanosuspension formulation for oral delivery of quercetin. *Journal of Biomedical Nanotechnology* **6**, 325–332.

Sundar, S. and Chakravarty, J. (2015). An update on pharmacotherapy for leishmaniasis. *Expert Opinion on Pharmacotherapy* **2**, 237–252.

Torres-Santos, E. C., Rodrigues, J. M., Moreira, D. L., Kaplan, M. A. C. and Rossi-Bergmann, B. (1999). Improvement of *in vitro* and *in vivo* antileishmanial activities of 2′,6′-dihydroxy-4′-methoxychalcone by entrapment in poly(D,L-lactide) nanoparticles. *Antimicrobial Agents and Chemotherapy* **43**, 1776–1778.

Tran, T. H., Guo, Y., Song, D., Bruno, R. S. and Lu, X. (2014). Quercetin-containing self-nanoemulsifying drug delivery system for improving oral bioavailability. *Journal of Pharmaceutical Sciences* **103**, 840–852.

Venturini, C. G., Jäger, E., Oliveira, C. P., Bernardi, A., Battastini, A. M. O., Guterres, S. S. and Pohlmann, A. R. (2011). Formulation of lipid core nanocapsules. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **375**, 200–208.

Weiss-Angeli, V., Poletto, F. S., De Marco, S. L., Salvador, M., Da Silveira, N. P., Guterres, S. S. and Pohlmann, A. R. (2012). Sustained antioxidant activity of quercetin-loaded lipid-core nanocapsules. *Journal of Nanoscience and Nanotechnology* **12**, 2874–2880.

World Health Organization (2015). *Investing to Overcome the Global Impact of Neglected Tropical Diseases: Third WHO Report on Neglected Tropical Diseases*. World Health Organization, Geneva, Switzerland.