Fullerenol C60(OH)24 nanoparticles decrease relaxing effects of dimethyl sulfoxide on rat uterus spontaneous contraction

Article *in* Journal of Nanoparticle Research · January 2013

CITATIONS
15

READS
198

8 authors, including:

Marija Slavic
University of Belgrade
20 PUBLICATIONS **225** CITATIONS
SEE PROFILE

Aleksandar Djordjevic
University of Novi Sad
124 PUBLICATIONS **2,913** CITATIONS
SEE PROFILE

Radomir Z. RadojičIć
52 PUBLICATIONS **1,056** CITATIONS
SEE PROFILE

Slobodan Milovanovic
University of East Sarajevo
10 PUBLICATIONS **182** CITATIONS
SEE PROFILE

J Nanopart Res (2013) 15:1650
DOI 10.1007/s11051-013-1650-1

RESEARCH PAPER

Fullerenol $C_{60}(OH)_{24}$ nanoparticles decrease relaxing effects of dimethyl sulfoxide on rat uterus spontaneous contraction

Marija Slavic · Aleksandar Djordjevic · Ratko Radojicic · Slobodan Milovanovic · Zorana Orescanin-Dusic · Zlatko Rakocevic · Mihajlo B. Spasic · Dusko Blagojevic

Received: 15 November 2012 / Accepted: 12 April 2013

Abstract Dimethyl sulfoxide (DMSO) is a widely used solvent and cryoprotectant that can cause impaired blood flow, reduction in intracranial pressure, tissue edema, inflammatory reactions, inhibition of vascular smooth muscle cell migration and proliferation, processes which can lead to atherosclerosis of the coronary, peripheral and cerebral circulation. Although the adverse effects are rare when DMSO is administered in clinically established concentrations, there is no safe antagonist for an overdose. In this work, we treated isolated spontaneous and calcium-induced contractile active rat uteri (Wistar, *virgo intacta*), with DMSO and fullerenol $C_{60}(OH)_{24}$ nanoparticle (FNP) in DMSO. FNP is a water-soluble derivative of fullerene C_{60}. Its size is a 1.1 nm in diameter and is a very promising candidate for a drug carrier in nanomedicine. FNP also displays free radical scavenging activity. DMSO decreased both spontaneous and calcium-induced contractions. In contrast, FNP only decreased spontaneous contraction. FNP decreased copper–zinc superoxide dismutase activity and prevented the DMSO-induced increase in glutathione reductase activity. Atomic force microscopy detected that FNP aggregated with calcium ions. Our results indicate that FNP has properties that make it a good candidate to be a modulator of DMSO activity which could minimize side effects of the latter.

Keywords DMSO · Fullerenol $C_{60}(OH)_{24}$ nanoparticles · AFM · Uterus · SOD · Glutathione

M. Slavic (✉) · Z. Orescanin-Dusic · M. B. Spasic · D. Blagojevic
Department for Physiology, Institute for Biological Research "Sinisa Stankovic" (IBISS), University of Belgrade, Bulevar Despota Stefana 142, 11000 Belgrade, Serbia
e-mail: marija17@ibiss.bg.ac.rs

A. Djordjevic
Department of Chemistry, Biochemistry and the Environment, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

R. Radojicic
Faculty of Biology, University of Belgrade, Belgrade, Serbia

S. Milovanovic
Department of Pharmacology, Faculty of Medicine at Foca, University of East Sarajevo, East Sarajevo, Republic of Srpska, Bosnia and Herzegovina

Z. Rakocevic
Institute for Nuclear Sciences "Vinca", University of Belgrade, Belgrade, Serbia

Published online: 21 April 2013

Introduction

Dimethyl sulfoxide (DMSO) is a small molecule with a hydrophilic sulfoxide group and two hydrophobic methyl groups that is widely used as polar aprotic solvent for water-insoluble chemicals. DMSO is used in medicine because of its analgesic and anti-inflammatory properties (Kloesch et al. 2011; Hollebeeck et al. 2011). In the United States, DMSO received approval from the FDA in 1978 for use in the treatment for interstitial cystitis by intravesicular administration (Parkin et al. 1997). It is also an effective penetration enhancer for many therapeutic drugs (Marren 2011), and it is successfully being used as a cryoprotectant. Regarding the latter, it has been found that DMSO enables cryoprotective solutions to solidify at very low temperatures without the formation of ice. This is important because ice-free cryopreservation is followed by a high rate of cell survival (Rall and Fahy 1985). However, before transplantation, it is necessary to remove DMSO from cryopreserved tissue samples to avoid toxicity caused to the host by the absorption of DMSO from the graft (Fahy 2010).

Fullerene C_{60} is the third allotropic form of elemental carbon. Its specific structure, which resembles a soccer ball, and its size (1.1 nm) together with its physical and chemical properties make this molecule a very promising candidate for a drug carrier in nanomedicine. However, because of its poor solubility in water and its toxic properties toward living cells, it lacks obvious employment in biology. Covalent addition of hydroxyl groups to the hydrophobic core significantly increases the solubility and changes the chemical properties of fullerene. By using water-soluble derivatives, many researchers have investigated their significance as prospective tools for nanomedicine. Fullerenol $C_{60}(OH)_{24}$ nanoparticles (FNP) are one group of a water-soluble derivative of fullerene C_{60} that have displayed a diverse range of biological activity. FNP does not elevate the frequency of chromosome aberrations (Mrdjanovic et al. 2009, 2012; Fenech 2002), and it can stabilize the DNA helix (Pinteala et al. 2009). FNP exhibits antioxidant activities and anti-inflammatory activities as well as the properties of a polydentate chelator (Dragojevic-Simic et al. 2011; Injac et al. 2008). Similar to non-hydroxylated water-soluble fullerene derivatives, the most important characteristics of FNP regarding mechanisms of their biological activity are their photosensitizing property and free radical scavenging activity. These properties are important not only in medicine (for example, disease treatment and disinfection) but also in environmental research (for example, acceleration of oxidative decomposition of organic compounds) (Brant et al. 2007; Partha and Conyers 2009).

In the last decade, there have been a growing number of experiments within the field of uterine transplantation. Although uterine transplantation is not a "life or death" situation, it could represent another therapeutic approach to restoration of fertility (Grynberg et al. 2011). The first human uterus transplantation was performed in 2000 but, unfortunately, after 99 days, it was necessary to perform a hysterectomy (Fageeh et al. 2002). It is hoped that in a few years, the success rate will have increased. Before such time, a suitable protocol for cryopreservation of reproductive organs should be established. The first successful cryopreservation of a whole pig uterus involved perfusion with DMSO followed by slow freezing of the organ (Dittrich et al. 2006). It has been reported that DMSO at a concentration commonly used in cryopreservation oxidizes glutathione both in vitro and in human erythrocytes. Although the rate of reaction is not fast, there is a substantial oxidation, and the rate of oxidation is dependent on DMSO concentration (Homer et al. 2005). This implies that cells and organs that undergo cryopreservation are at risk because of the redox imbalance. An elevated GSSG/GSH ratio may cause oxidative stress as GSH is a thiol substrate for enzymes that reduce free radicals and also GSSG is very toxic *per se*. Homeostasis of thiol glutathione is also essential for normal embryo development (Salmen et al. 2005). Transplantation and pregnancy themselves can trigger elevation of reactive oxygen species. Hence, the depletion of thiol glutathione may cause an additional oxidative damage in embryo. Bearing in mind the effects of DMSO, it is of great importance to have a substance that could modulate actions of DMSO and potentiate its beneficial properties. The aim of our study was to investigate effects of DMSO on uterine smooth muscle activity and the status of antioxidative enzymes in uterus. We also examined the potential role of FNP in modulating DMSO's effects on enzymes activities.

Materials and methods

Materials

Fullerenol $C_{60}(OH)_{24}$ was synthesized in alkaline media by complete substitution of bromine atoms

from $C_{60}Br_{24}$ (Djordjevic et al. 1998; Mirkov et al. 2004), while DMSO was commercially available (99.9 %,Carlo Erba, Italy). The following were used: DMSO with FNP (final concentrations): 2.5, 5, 10, 30, 70, 150, 250, 350, 450 μg/mL; DMSO: 7.04, 14.08, 28.16, 84.48, 197.12, 422.4, 704, 985.6 mM and 1.27 M. De Jalon's solutions contained (in g/L): NaCl 9.0, KCl 0.42, $NaHCO_3$ 0.5, $CaCl_2$ 0.06 and glucose 0.5.

Atomic force microscopy

Morphology and structure of (1) DMSO with FNP and (2) DMSO with FNP in De Jalon's solution were evaluated using atomic force microscopy (AFM). Surface topography and phase images were simultaneously acquired by standard AFM tapping mode using a commercial SNC (Solid Nitride Cone) AFM probe (NanoScience-Team Nanotec GmbH), with the tip radius lower than 10 nm. Highly orientated pyrolytic graphite (HOPG) was used as surface. Multimode quadrex SPM with a Nanoscope IIIa controller (Veeco Instruments, Inc.) operated under ambient conditions was used.

Experimental system

All protocols for handling rats were approved by the local Ethics Committee for Animal Experimentation that strictly follows international regulations. Animals were kept at 22 °C, housed 3 per cage and fed ad libitum. Our model system was isolated uteri from 10-week-old virgin Wistar rats (200–250 g) in estrous phase of the ovarian cycle, as determined by the examination of a daily vaginal lavage (Marcondes et al. 2002).

Isolated organ bath studies

All rats were decapitated by cervical dislocation. The uterine horns were rapidly excised and carefully cleaned of surrounding connective tissue and mounted vertically in a 10-mL volume organ bath containing De Jalon's solution (NaCl 154 mM, KCl 5.6 mM, $CaCl_2$ x $2H_2O$ 0.41 mM, $NaHCO_3$ 5.9 mM and glucose 2.8 mM) for 2 h, under 1 g tension, aerated with 95 % oxygen and 5 % carbon dioxide at 37 °C. The uteri, both spontaneously active and calcium-ion (Ca^{2+})-induced, were allowed to stabilize their contractions (for about 20 min) before addition of experimental drugs. There were adequate controls for each type of uterine activity. A control for spontaneous activity (C_{sp}) comprised untreated uteri incubated in De Jalon's solution for equivalent experimental time (2 h) in the same experimental conditions. A control group for Ca^{2+}-induced activity comprised uteri activated with 6 mM Ca^{2+} and incubated for 2 h in the same experimental conditions (C_{ca}). DMSO was added cumulatively with final concentrations: 7.04, 14.08, 28.16, 84.48, 197.12, 422.4, 704, 985.6 mM and 1.27 M. DMSO with FNP was added cumulatively with final concentrations of FNP: 2.5, 5, 10, 30, 70, 150, 250, 350, 450 μg/mL. When uteri achieved stable contractions (spontaneous or Ca^{2+}-induced), myometrial tension was recorded isometrically with a TSZ-04-E isolated organ bath and transducer (Experimetria, Budapest, Hungary). Seven to thirteen uteri were used per experiment. The number of uteri (n) used for each experiment is given in the figure legends.

After treatment, samples were immediately frozen using liquid nitrogen and then transferred to −80 °C until analysis.

Determination of antioxidant enzyme activities

Thawed uteri were homogenized and sonicated in 0.25 M sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.05 M Tris–HCl buffer pH 7.4 before centrifugation for 90 min at 105,000×*g*. The supernatant was used to determine the enzyme activities using a Shimadzu UV-160 spectrophotometer (Shimadzu Scientific Instruments, Shimadzu Corporation, Kyoto, Japan). Superoxide dismutase (SOD) activities were determined by the adrenaline method (Misra and Fridovich 1972). One unit of activity is defined as the amount of enzyme necessary to decrease the rate of adrenalin auto-oxidation by 50 % at pH 10.2. Manganese SOD (MnSOD) activity was determined by incubating the samples with 8 mM KCN. CuZnSOD activity was calculated as the difference between total SOD and MnSOD activities. The activity of glutathione peroxidase (GSH-Px) was determined by the GSH-dependent reduction in *t*-butyl hydroperoxide using a modification of the assay described by Paglia and Valentine (1967). One unit of GSH-Px activity is defined as the amount needed to oxidize 1 nmol NADPH per min at 25 °C and pH 7.0.

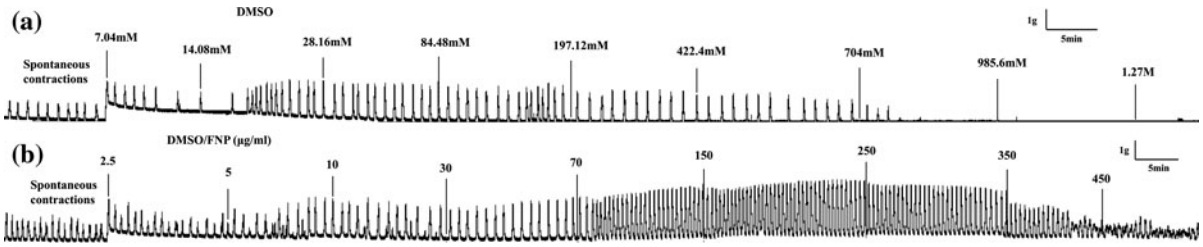

Fig. 1 A representative original trace of spontaneous uterine contractions treated with cumulative concentrations: **a** DMSO: 7.04, 14.08, 28.16, 84.48, 197.12, 422.4, 704, 985.6 mM and 1.27 M and **b** DMSO with FNP (final concentrations of FNP were 2.5, 5, 10, 30, 70, 150, 250, 350, 450 μg/mL). Abbreviations: FNP, fullerenol $C_{60}(OH)_{24}$ nanoparticles; DMSO, dimethyl sulfoxide

GR activity was determined using the method of Glatzle et al. (1974). This assay is based on NADPH oxidation concomitant with GSH reduction. One unit of GR activity is defined as the oxidation of 1 nmol NADPH per min at 25 °C and pH 7.4. All enzyme activities were expressed as units × mg^{-1} protein.

Data analysis and statistical procedures

Statistical analyses (descriptive statistics, analysis of variance—ANOVA and *F*-test) were performed according to protocols described by Hinkle et al. (1994) and Manley (1986) using Statistical Analysis Software, version 9.1.3 (SAS Institute Inc., NC, USA). The effects of treatments on uterine contractions were calculated as the percentage of control, untreated, contractions. All data are expressed as the mean ± SEM or SD (type of the error presented is indicated in every figure legend). Differences between groups were tested by two-way ANOVA with treatment and dose as factors and were considered statistically significant when $p < 0.05$. Dose–response curves were sigmoidal in shape fitted to Boltzmann functions (the concentration axis was linear). Sigmoid curves were compared using the *F*-test. The activity of antioxidant enzymes was compared using one-way ANOVA followed by a Tukey's HSD post hoc test (significance $p < 0.05$).

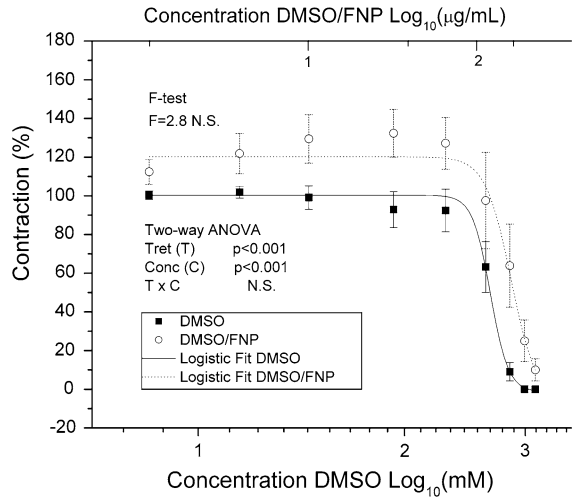

Fig. 2 Spontaneous contractile activity of rat uteri treated with DMSO: 7.04, 14.08, 28.16, 84.48, 197.12, 422.4, 704, 985.6 mM and 1.27 M; and DMSO with FNP (final concentrations of FNP were 2.5, 5, 10, 30, 70, 150, 250, 350, 450 μg/mL). *FNP* fullerenol $C_{60}(OH)_{24}$ nanoparticles, *DMSO* dimethyl sulfoxide. Data were analyzed by two-way ANOVA with treatment (T) and concentration (C) as factors ($p < 0.05$ was considered significant; *NS* nonsignificant). Data are expressed as mean ± SEM ($n = 8$). Both DMSO and DMSO with FNP significantly inhibited spontaneous contractions of rat uteri (ANOVA significant effect of concentration (C), $p < 0.001$). FNP dissolved in DMSO significantly reduce DMSO relaxant effect (ANOVA significant treatment effect (T), $p < 0.001$). Afterward, *sigmoidal fit curves* were compared with *F*-test. There were no statistical differences between the *curves* ($F = 2.8$, N.S)

Results

Contractions of isolated rat uteri

DMSO caused significant relaxation of both spontaneously and Ca^{2+}-induced contractions (Figs. 1a, 3a). In spontaneously active uteri, cumulative doses of DMSO with FNP significantly reduced the relaxant effect of DMSO (Figs. 1b, 2), while in Ca^{2+}-induced uteri DMSO with FNP did not affect the relaxant effect of DMSO (Figs. 3b, 4).

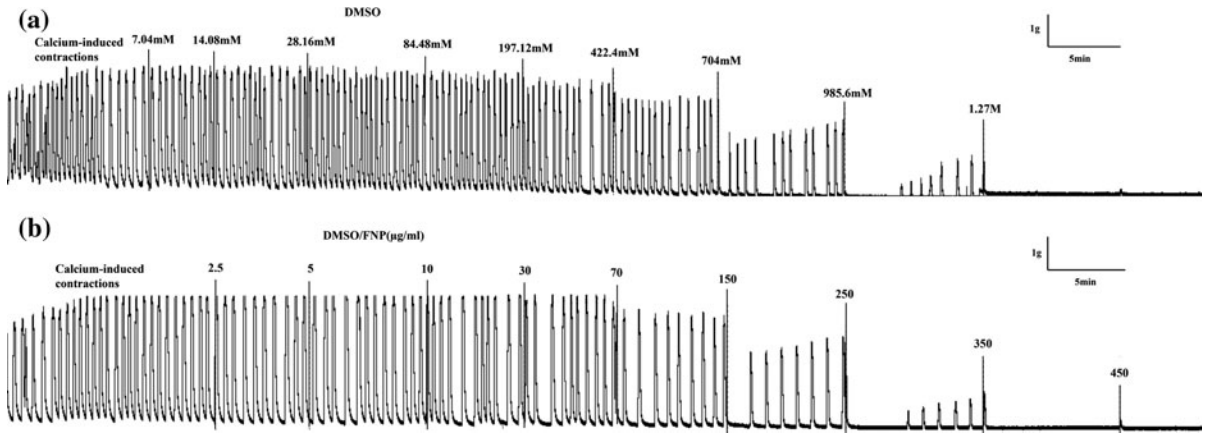

Fig. 3 A representative original trace of Ca^{2+}-induced uterine contractions treated with cumulative concentration: **a** DMSO: 7.04, 14.08, 28.16, 84.48, 197.12, 422.4, 704, 985.6 mM and 1.27 M and **b** DMSO with FNP (final concentrations of FNP were 2.5, 5, 10, 30, 70, 150, 250, 350, 450 μg/mL). *FNP* fullerenol $C_{60}(OH)_{24}$ nanoparticles, *DMSO* dimethyl sulfoxide

AFM study

Figures 5 and 6 present AFM images of (1) DMSO with FNP and (2) DMSO/FNP in De Jalon's solution after 2 h incubation at 37 °C.

DMSO with FNP formed a non-homogeneous system on HOPG surface (Fig. 5). Dominant nanoaggregates were dimmers with a diameter of approximately 90 nm. Subunits were 50 and 40 nm in diameter, and they were composed of smaller particles of about 20 and 30 nm in diameter. Particles larger than 200 nm in diameter were not present in as significant percentage. The majority of fullerenol nanoparticles were retained on the HOPG terraces, indicating that FNP had hydrophilic properties.

When the DMSO with FNP was added into organ baths containing De Jalon's solution, a brown precipitate formed. Every subsequent dose was followed by a larger amount of precipitate. Fig. 6 presents AFM measurements of DMSO with FNP in De Jalon's solution. FNP, as polyanionic polydentate ligand, formed networked nanocomposites with Ca^{2+} from De Jalon's solution. The formation of such composite required a concentration range of 10^{-3}–10^{-5} M Ca^{2+}. The AFM image shows a non-homogeneous system of FNP- Ca^{2+} networked nanocomposite.

Anti-oxidative enzyme activities

In both spontaneous and Ca^{2+}-induced contracting uteri, DMSO with FNP significantly decreased CuZn-SOD activity compared to adequate control (Figs. 7, 8).

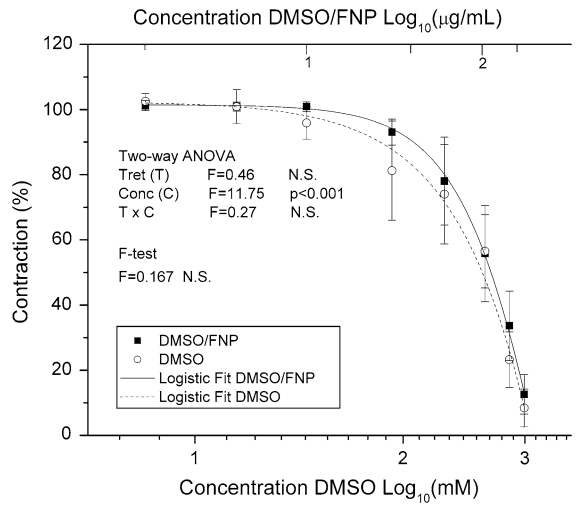

Fig. 4 Activity of Ca^{2+}-induced uterine contractions treated with DMSO: 7.04, 14.08, 28.16, 84.48, 197.12, 422.4, 704, 985.6 mM and 1.27 M and DMSO with FNP (final concentrations of FNP were 2.5, 5, 10, 30, 70, 150, 250, 350, 450 μg/mL). Data were analyzed by two-way ANOVA with treatment (T) and concentration (C) as factors ($p < 0.05$ was considered significant; *NS* nonsignificant). Data are expressed as mean ± SEM ($n = 7$). Both DMSO and DMSO with FNP significantly inhibited spontaneous contractions of rat uteri (ANOVA significant effect of concentration (C), $p < 0.001$). Effect of DMSO with FNP on Ca^{2+}-induced uterine contractions was not statistically different from the effect of DMSO on Ca^{2+}-induced uterine contractions (no significant ANOVA treatment effect (T), NS). Afterward, the *sigmoidal fit curves* were compared with *F*-test. There were no statistical differences between the *curves* ($F = 0.167$, N.S)

There were no changes in MnSOD activity in any of the groups of uteri. DMSO significantly increased the activity of GR in spontaneously active uteri (Fig. 7),

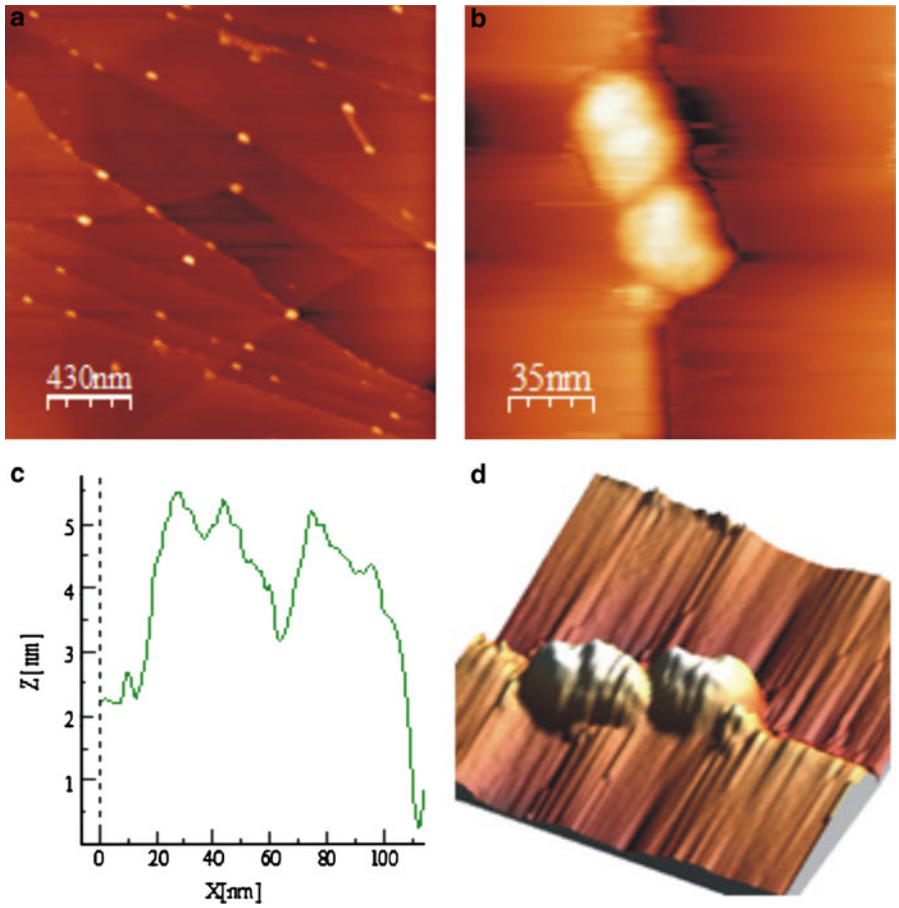

Fig. 5 AFM images of DMSO with FNP after 2 h at 37 °C **a** large-scale image, 2,000 × 2,000 nm^2, and **b** small-scale image, 170 × 170 nm^2, of nanoparticles of about 90 nm on HOPG surface; **c** corresponding cross section of fullerenol nanoparticles. Maximal peak of the particle was 5.5 nm; smaller particle peak was 4.5 nm, respectively; **d** 3D image of FNP on the HOPG surface

while DMSO with FNP decreased the DMSO-induced elevation of GR in spontaneously active uteri (Fig. 7). Changes in GR activity were not followed by significant changes in GSH-Px activity in any of the groups.

Data were analyzed by two-way ANOVA with treatment (T) and concentration (C) as factors ($p < 0.05$ was considered significant; NS—non significant). Data are expressed as mean ± SEM ($n = 7$). Both DMSO and DMSO with FNP significantly inhibited spontaneous contractions of rat uteri (ANOVA significant effect of concentration (C), $p < 0.001$). However, DMSO with FNP partially reversed DMSO-mediated inhibition. The effect of DMSO with FNP on Ca^{2+}-induced uterine contractions was not statistically different from the effect of DMSO on Ca^{2+}-induced uterine contractions [no significant ANOVA treatment effect (T), NS]. After the sigmoidal fit curves were compared with F-test, there were no statistical differences between the curves ($F = 0.167$, NS).

Discussion

DMSO has a relaxant effect on both types (spontaneous and Ca^{2+}-induced) of smooth muscle activity investigated in our experiments. It has been demonstrated that DMSO induces relaxation in the rabbit detrusor muscle by decreasing the Ca^{2+} sensitivity of the contractile apparatus mainly because of the inhibition of myosin light chain phosphorylation. However, the intracellular level of Ca^{2+} was not affected (Shiga et al. 2007). Inhibition of cross-bridge cycling by obstruction of phosphate release may have also been involved in the relaxant effect of DMSO (Mariano et al. 2001).

Our results showed that FNP significantly reduces the relaxant effect of DMSO on spontaneously active uterine smooth muscle (Figs. 1, 2). Other fullerene derivatives tested on smooth muscle activity inhibited endothelium-dependent relaxation induced by acetylcholine (Satoh and Takayanagi 2006). Further investigations confirmed

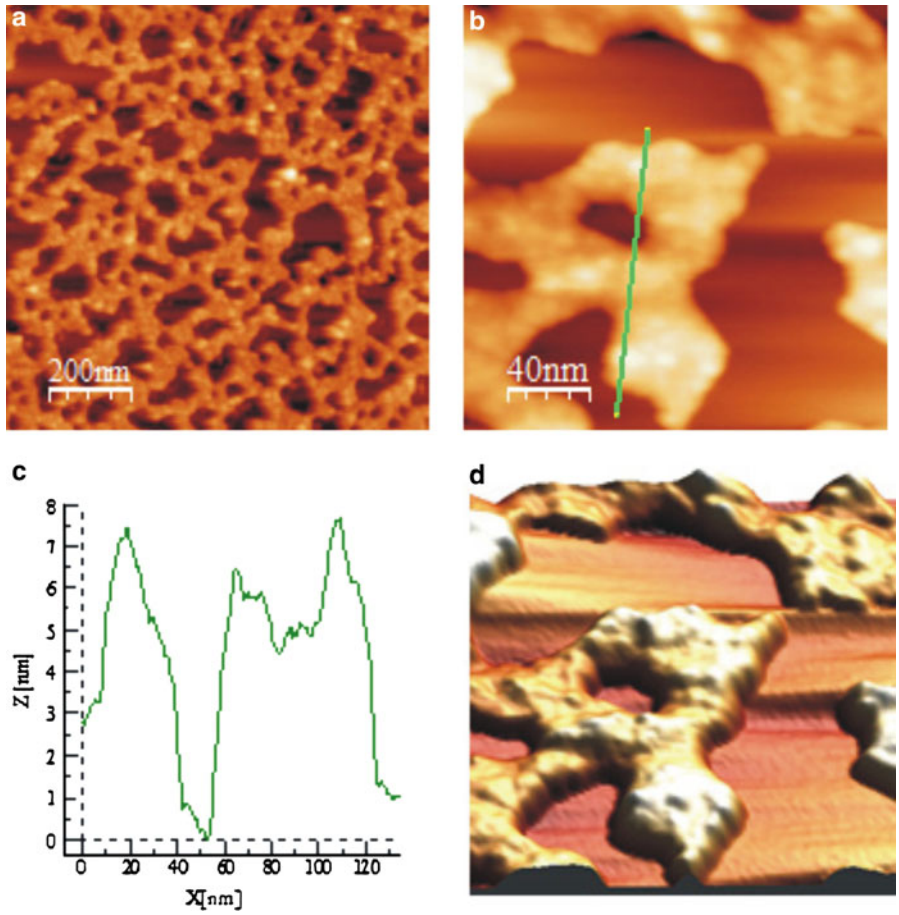

Fig. 6 AFM image of network of DMSO with FNP added to De Jalon's solution **a** large-scale image, 1,000 × 1,000 nm^2, and **b** small-scale image, 200 × 200 nm^2, of FNP-Ca^{2+} networked nanocomposite; **c** corresponding cross section of FNP-Ca^{2+} networked nanocomposite. Maximal peak of the networked nanocomposite was 8.0 nm; smaller peak was 6.5 nm, respectively; **d** 3D image of FNP-Ca^{2+} network nanocomposite on the HOPG surface

endothelial integrity in treatment with fullerene derivatives as well as restoration of sensitivity to acetylcholine after the addition of superoxide dismutase. It has been assumed that water-soluble fullerene derivatives disable the relaxant effect of free- and agonist-induced production of nitric oxide (Satoh and Takayanagi 2006). This could be one of the possible ways by which the FNP reduces the relaxant effect of DMSO as DMSO, among its other effects, also induces the release of nitric oxide (Birder et al. 1997).

According to AFM, DMSO probably built some kind of thread over the basic motif of FNP nanoaggregates, thereby enhancing the transport of FNP into tissue or at least, delays its complete precipitation. In De Jalon's solution, FNP aggregated with alkaline earth metals (mostly Ca^{2+}) over 12 h, which gave FNP enough time to exhibit at least a dual effect. The first was the interaction with tissue, and the second was the lowering of the actual concentration alkaline metals in De Jalon's solution AFM showed FNP-Ca^{2+} nanocomposite formation in De Jalon's solution (Fig. 6). Polyanion FNP nanoaggregates are surrounded and interconnected with Ca^{2+} ions, and they build a network of FNP-Ca^{2+} nanocomposites. An interesting investigation of similar hybrid microspheres calcium carbonate/fullerenol has previously been reported (Calvaresi et al. 2011). Such a model could be an efficient drug delivery system. Gelderman and colleagues investigated the relationship between intracellular Ca^{2+} and cell treatment with both FNP and fullerene. Both increased the intracellular level of Ca^{2+}. However, EGTA blocked this effect, which implies that during treatment with these nanoparticles, Ca^{2+} enters from the extracellular space, either by activation of calcium channels or by enlarging pores within cell membrane which additionally enables calcium ions to enter the cell (Gelderman et al. 2008).

DMSO caused significant relaxation of Ca^{2+}-induced uterine contractions (Fig. 3a). However, FNP failed to reduce this effect like in spontaneous

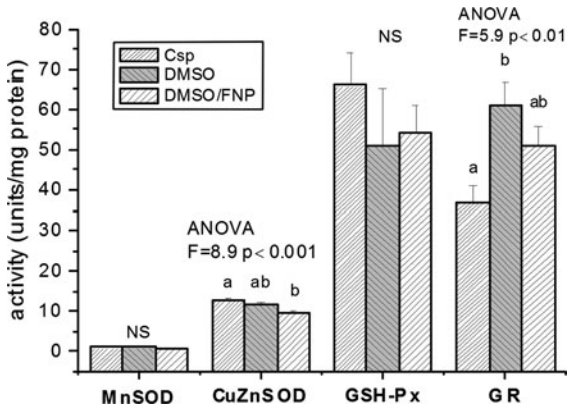

Fig. 7 Antioxidant enzyme activities in spontaneously active rat uteri. Enzyme activities were determined in untreated rat uteri incubated for 2 h at 37 °C (C_{sp}, $n = 13$), rat uteri incubated for 2 h at 37 °C treated with increasing concentration of DMSO ($n = 8$) and rat uteri incubated for 2 h at 37 °C treated with increasing concentration of DMSO with FNP (DMSO/FNP, $n = 10$). Concentrations were given above in Fig. 1. Data are expressed as mean ± SD. The groups were compared with one-way ANOVA ($p < 0.05$ was considered significant; *NS* nonsignificant) followed by post hoc Tukey's HSD test for unequal number of samples (n). F values are presented as well as probability levels to denote differences. *Different letters* above *error bars* denote the difference between individual groups

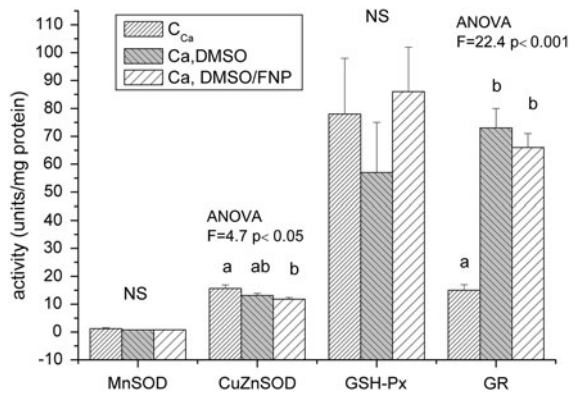

Fig. 8 Antioxidant enzyme activities in Ca^{2+}-induced uterine contractions. Enzyme activities were determined in rat uteri activated with 6 mM Ca^{2+} incubated for 2 h at 37 °C (C_{ca}, $n = 5$), rat uteri incubated for 2 h at 37 °C treated with increasing concentration of DMSO ($n = 8$) and rat uteri incubated for 2 h at 37 °C treated with increasing concentration of DMSO with FNP (DMSO/FNP, $n = 9$). Concentration was given above in Fig. 3. Data are expressed as mean ± SD. The groups were compared with one-way ANOVA ($p < 0.05$ was considered significant; *NS* nonsignificant) followed by post hoc Tukey's HSD test for unequal number of samples (n). F values are presented as well as probability levels to denote differences. *Different letters* above *error bars* denote the difference between individual groups

uterine contractions (Figs. 3b, 4). Large quantities of exogenous Ca^{2+} entered the cells through Ca^{2+} channels during Ca^{2+}-induced uterine activity, so the effect of complex of FNP-Ca^{2+} could not be perceived.

In both types of uterine contractions, DMSO decreased CuZnSOD activity to some extent while FNP significantly decreased its activity (Figs. 7, 8). When not photoexcited, FNP acts as a superoxide anion scavenger. In other words, it competes with endogenous superoxide dismutase for the same substrate (Mirkov et al. 2004). The study of Ali and co-workers (Ali et al. 2004) supports our result. Ali and co-workers found that the water-soluble *tris*-malonic acid derivative of fullerene C_{60} (C_3) displays SOD-mimetic properties. This catalytic reaction is slower than the reaction mediated by SOD, but it also generates hydrogen peroxide (H_2O_2) and molecular oxygen. Additionally, the authors suggested that C_3 also interacts with H_2O_2 (Ali et al. 2004). Fullerenol has been shown to block H_2O_2-induced inhibition of population spikes in rat hippocampus (Tsai et al. 1997). Our recent findings showed that H_2O_2 had a relaxant effect on uterine smooth muscle contractions (Appiah et al. 2009). Besides protecting the cell by scavenging H_2O_2, the interaction between FNP and H_2O_2 could also be an additional mechanism by which FNP reduces relaxation of spontaneously active uterine smooth muscle. Pertinent to this, there was no significant change in GSH-Px activity in our experiments, since the substrate for this enzyme is H_2O_2 (Figs. 7, 8).

The activity of glutathione reductase (GR) was increased in both spontaneous and Ca^{2+}-induced uterine smooth muscle, when treated with DMSO. No change in GSH-Px activity was found (Figs. 7, 8). The substrate for GR is an oxidized form of glutathione (GSSG). Elevation of the GSSG/GSH ratio is a response to a disturbed redox homeostasis in the cell. Although FNP exhibited a similar effect compared to control, in spontaneous uteri, FNP reduced the level of GR increased in the presence of DMSO in tissue (Fig. 7). The cumulative dose of DMSO used in our experiment (1.27 M) was close to the DMSO dose used in medium for cryopreservation ($\sim$1.4 M). Since it has been shown that in doses close to those in cryopreservation medium, DMSO can oxidize GSH in

both human erythrocytes and in vitro, our result may suggest that FNP acts as a modulator of DMSO effects in medicine as well as in biology. DMSO has been described as a universal cure, but there is no adequate modulator of DMSO activity that could minimize its side effects. Our results indicate that FNP has properties that make this nanoparticle a good candidate to be a modulator of DMSO activity that could minimize its side effects. Furthermore, we have demonstrated that apart from SOD-like activity, FNP also modulates the activity of GR. It is of interest in cryopreservation to maintain the GSH level in medium as this tripeptide is the main keeper of redox homeostasis. Pertinent to uterine transplantation, GSH is necessary for embryo development in the first trimester of pregnancy.

Acknowledgments This study was supported by a grant from the Ministry of Education, Science and Technological Development of the Republic of Serbia, project No 173014B: "Molecular mechanisms of redox signalling in homeostasis: adaptation and pathology" and grant No III45005: "Functional, functionalized and accomplished nanomaterials."

References

Ali SS, Hardt JI, Quich KL, Kim-Han JS, Erlanger BF, Huang TT, Epstein CJ, Dugan LL (2004) A biologically effective fullerene (C_{60}) derivative with superoxide dismutase mimetic properties. Free Radic Biol Med 37(8):1191–1202. doi:10.1016/j.freeradbiomed.2004.07.002

Appiah I, Milovanovic S, Radojicic R, Nikolic-Kokic A, Orescanin-Dusic Z, Slavic M, Trbojevic S, Skrbic R, Spasic MB, Blagojevic D (2009) Hydrogen peroxide affects contractile activity and anti-oxidant enzymes in rat uterus. Br J Pharmacol 158(8):1932–1941. doi:10.1111/j.1476-5381.2009.00490.x

Birder LA, Kanai AJ, de Groat WC (1997) DMSO: effect on bladder afferent neurons and nitric oxide release. J Urol 158:1989–1995. doi:10.1016/S0022-5347(01)64199-5

Brant JA, Labile J, Robichaund CO, Wiesner M (2007) Fullerol cluster formation in aqueous solutions: implications for environmental release. J Colloid Interface Sci 314:281–288. doi:10.1016/j.jcis.2007.05.020

Calvaresi M, Falini G, Bonacchi S, Genovese D, Fermani S, Montalti M, Prodi L, Zerbetto F (2011) Fullerenol entrapment in calcite microspheres. Chem Commun (Camb) 47(38):10662–10664. doi:10.1039/C1CC13680A

Dittrich R, Maltaris T, Mueller A, Dimmler A, Hoffmann I, Kiesewetter F, Beckmann MW (2006) Successful uterus cryopreservation in an animal model. Horm Metab Res 38(3):141–145. doi:10.1055/s-2006-925175

Djordjevic A, Vojinovic-Mloradov M, Petranovic N, Devcerski A, Layar D, Ribar B (1998) Catalytical preparation and characterization of S60Br24. Fullerene Sci Technol 6:689–694. doi:10.1080/10641229809350229

Dragojevic-Simic V, Jacevic V, Dobric S, Djordjevic A, Bokonjic D, Bajcetic M, Injac R (2011) Anti-inflammatory activity of fullerenol $C_{60}(OH)_{24}$ nano-particles in a model of acute inflammation in rats. Dig J Nanomater Biostruct 6:819–827

Fageeh W, Raffa H, Jabbad H, Marzouki A (2002) Transplantation of the human uterus. Int J Gynecol Obstet 76(3):245–251. doi:10.1016/S0020-7292(01)00597-5

Fahy GM (2010) Cryoprotectant toxicity neutralization. Cryobiology 60:S45–S53

Fenech M (2002) Chromosomal biomarkers of genomic instability relevant to cancer. Drug Discov Today 7(22):1128–1137

Gelderman MP, Simakova O, Clogston JD, Patri AK, Siddiqui SF, Vostal AC, Simak J (2008) Adverse effect of fullerenes on endothelial cells: fullerenol $C_{60}(OH)_{24}$ induced tissue factor and ICAM-1 membrane expression and apoptosis in vitro. Int J of Nanomed 3(1):59–68

Glatzle D, Vuilleumier JP, Weber F, Decker K (1974) Glutathione reductase test with whole blood a convenient procedure for the assessment of the riboflavin status in humans. Experimetria 30:665–668

Grynberg M, Ayoubi J-M, Bulletti C, Frydman R, Franchin R (2011) Uterine transplantation: a promising surrogate to surrogacy? Reprod Sci 1221:47–53

Hinkle ED, Wiersma W, Jurs GS (1994) Applied statistics for behavioral sciences. Houghton Mifflin Company, Boston

Hollebeeck S, Raas T, Piront N, Schneider YJ, Toussaint O, Larondelle Y, During A (2011) Dimethyl sulfoxide (DMSO) attenuates the inflammatory response in the in vitro intestinal Caco-2 cell model. Toxicol Lett 206(3):268–275. doi:10.1016/j.toxlet.2011.08.010

Homer ZN, Reglinski J, Sowden R, Spickett CM, Wilson R, Walker JJ (2005) Dimethylsulfoxide oxidizes glutathione in vitro and in human erythrocytes: kinetic analysis by ^{1}H NMR. Cryobiology 50:317–324. doi:10.1016/j.cryobiol.2005.04.002

Injac R, Radic N, Govedarica B, Djordjevic A, Strukelj B (2008) Bioapplication and activity of fullerenol $C_{60}(OH)_{24}$. Afr J Biotechnol 7(25):4050–4940

Kloesch B, Liszt M, Broell J, Steiner G (2011) Dimethyl sulphoxide and dimethyl sulphone are potent inhibitors of IL-6 and IL-8 expression in the human chondrocyte cell line C-28/I2. Life Sci 89(13-14):473–478. doi:10.1016/j.lfs.2011.07.015

Manley BFJ (1986) Multivariate statistical methods. Chapman & Hall, London

Marcondes FK, Bianchi FI, Tanno AP (2002) Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Biol 62:609–614. doi:10.1590/S1519-69842002000400008

Mariano AC, Alexandre GM, Silva LC, Romeiro A, Cameron LC, Chen Y, Chase PB, Sorenson MM (2001) Dimethyl sulphoxide enhances the effects of P(i) in myofibrils and inhibits the activity of rabbit skeletal muscle contractile proteins. Biochem J 358:627–636. doi:10.1042/0264-6021:3580627

Marren K (2011) Dimethyl sulfoxide: an effective penetration enhancer for topical administration of NSAIDs. Phys Sportsmed 39(3):75–82. doi:10.3810/psm.2011.09.1923

Mirkov SM, Djordjevic AN, Andric NL, Andric SA, Kostic TS, Bogdanovic GM, Vojinovic-Miloradov MB, Kovacevic RZ (2004) Nitric oxide-scavenging activity of polyhydroxylated fullerenol, $C_{60}(OH)_{24}$. Nitric Oxide 11(2): 201–207

Misra HP, Fridovich I (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247(10):3170–3175

Mrdjanovic J, Solajic S, Bogdanovic V, Stankov K, Bogdanovic G, Djordjevic A (2009) Effects of fullerenol $C_{60}(OH)_{24}$ on the frequency of micronuclei and chromosome aberrations in CHO-K1 cells. Mutat Res 680:25–30

Mrdjanovic JZ, Solajic SV, Bogdanovic VV, Stankov K, Djordjevic AN, Bogdanovic GM, Injac RD, Rakocevic ZLJ (2012) Effects of fullerenol nano particles C_{60} $(OH)_{24}$ on micronuclei and chromosomal aberrations' frequency in peripheral blood lymphocytes. Dig J Nanomater Bios 7(2):673–686

Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70:74–77

Parkin J, Shea C, Sant GR (1997) Intravesical dimethyl sulfoxide (DMSO) for interstitial cystitis-a practical approach. Urology 49(5, Suppl):105–107. doi:10.1016/S0090-4295(97)00181-7

Partha R, Conyers JL (2009) Biomedical applications of functionalized fullerene-based nanomaterials. Int J Nanomed 4:261–275

Pinteala M, Dascalu A, Ungurenasu C (2009) Binding fullerenol $C_{60}(OH)_{24}$ to dsDNA. Int J Nanomed 4:193–199

Rall WF, Fahy GM (1985) Ice-free cryopreservation of mouse embryos at −196 degrees C by vitrification. Nature 313 (6003):573–575. doi:10.1038/313573a0

Salmen JJ, Skufca F, Matt A, Gushansky G, Mason A, Gardiner CS (2005) Role of glutathione in reproductive tract secretion on mouse preimplantation embryo development. Biol Reprod 73:308–314. doi:10.1095/biolreprod.104.038307

Satoh M, Takayanagi I (2006) Pharmacological studies on fullerene (C_{60}), a novel carbon allotrope, and its derivatives. J Pharmacol Sci 100:513–518. doi:10.1254/jphs.CPJO6OO2X

Shiga KI, Hirano K, Nishimura J, Niiro N, Kanaide H (2007) Dimethyl sulphoxide relaxes rabbit detrusor muscle by decreasing the Ca^{+2} sensitivity of the contractile apparatus. Br J Pharmacol 151(7):1014–1024

Tsai MC, Chen YH, Chiang LY (1997) Polyhydroxylated C60 fullerenol, a novel free radical trapper, prevented hydrogen peroxide- and cumene hydroperoxide-elicited changes in rat hippocampus in vitro. J Pharm Pharmacol 49:438–445. doi:10.1111/j.2042-7158.1997.tbO6821.x