Anxiolytic-like effect of oxytocin in the simulated public speaking test
Anxiolytic-like effect of oxytocin in the simulated public speaking test
Danielle CG de Oliveira, Antonio W Zuardi, Frederico G Graeff, Regina HC Queiroz and José AS Crippa

J Psychopharmacol 2012 26: 497 originally published online 9 May 2011
DOI: 10.1177/0269881111400642

The online version of this article can be found at:
http://jop.sagepub.com/content/26/4/497

Published by:
SAGE
http://www.sagepublications.com

On behalf of:
British Association for Psychopharmacology

Additional services and information for Journal of Psychopharmacology can be found at:

Email Alerts: http://jop.sagepub.com/cgi/alerts
Subscriptions: http://jop.sagepub.com/subscriptions
Reprints: http://www.sagepub.com/journalsReprints.nav
Permissions: http://www.sagepub.com/journalsPermissions.nav

>> Version of Record - Apr 20, 2012
OnlineFirst Version of Record - May 9, 2011
What is This?
Anxiolytic-like effect of oxytocin in the simulated public speaking test

Danielle CG de Oliveira¹, Antonio W Zuardi¹,², Frederico G Graeff¹, Regina HC Queiroz²,³ and José AS Crippa¹,²

Abstract
Oxytocin (OT) is known to be involved in anxiety, as well as cardiovascular and hormonal regulation. The objective of this study was to assess the acute effect of intranasally administered OT on subjective states, as well as cardiovascular and endocrine parameters, in healthy volunteers (n = 14) performing a simulated public speaking test. OT or placebo was administered intranasally 50 min before the test. Assessments were made across time during the experimental session: (1) baseline (t = 0 min); (2) pre-test (t = 15 min); (3) anticipation of the speech (t = 50 min); (4) during the speech (t = 1:03 h), post-test time 1 (t = 1:26 h), and post-test time 2 (t = 1:46 h). Subjective states were evaluated by self-assessment scales. Cortisol serum and plasma adrenocorticotropic hormone (ACTH) were measured. Additionally, heart rate, blood pressure, skin conductance, and the number of spontaneous fluctuations in skin conductance were measured. Compared with placebo, OT reduced the Visual Analogue Mood Scale (VAMS) anxiety index during the pre-test phase only, while increasing sedation at the pre-test, anticipation, and speech phases. OT also lowered the skin conductance level at the pre-test, anticipation, speech, and post-test 2 phases. Other parameters evaluated were not significantly affected by OT. The present results show that OT reduces anticipatory anxiety, but does not affect public speaking fear, suggesting that this hormone has anxiolytic properties.

Keywords
Anxiety, healthy volunteers, oxytocin, simulated public speaking

Introduction
Oxytocin (OT) is a nonapeptide synthesized in the paraventricular and supraoptic nuclei of the hypothalamus. OT is transported in vesicles from the magnocellular nuclei of the supraoptic and paraventricular nuclei to the neurohypophysis, where it is stored and then released into the bloodstream. OT is also present in the central nervous system, where it originates from both the parvocellular and magnocellular neurons after dendritic local release (Bujis, 1978; Landgraf and Neumann, 2004; Ludwig et al., 2002).

OT has both peripheral endocrine and central neural actions. Its peripheral actions on lactation and parturition have long been established. Centrally, it has been suggested that OT acts as a neurotransmitter or neuromodulator regulating neuroendocrine, behavioral and autonomic responses to stress. Thus, it has been reported that OT administration attenuates glucocorticoid secretion (Ditzen et al., 2009; Heinrichs et al., 2003; Neumann, 2002; Neumann et al., 2000; Windle et al., 1997) and stress-induced increases in arterial pressure (Holst et al., 2002; Windle et al., 1997). In addition, OT had anxiolytic-like effects in both male (Ring et al., 2006; Waldherr et al., 2007) and female rats (Bale et al., 2001; Neumann et al., 2000; Windle et al., 1997) and mice (McCarthy et al., 1996; Mantella et al., 2004). Accordingly, Mantella et al. (2003) have shown that female OT knockout mice were more anxious than wildtype controls, an effect that was reversed by the central administration of OT. In addition, control mice showed increased anxiety after administration of an OT receptor antagonist. So far, studies in humans are scarce, but preliminary data suggest that intranasally administered OT has an anxiolytic-like effect (Heinrichs et al., 2003).

OT has also been shown to be involved in the regulation of social behavior. In monogamous female prairie voles (Microtus ochrogaster), OT is believed to participate in bond formation between couples, since this bonding is inhibited by a selective OT receptor antagonist (Insel and Hulihan, 1995; Williams et al., 1994). In humans, two recent studies...
used functional magnetic resonance imaging to assess the effect of OT on activation of the amygdala during recognition of emotional faces and in response to non-social stimuli (Domes et al., 2007; Kirsch et al., 2005). In the first study, the results showed decreased amygdala activation in response to negative social and non-social stimuli when OT was administered, relative to baseline. These results may explain why OT attenuates stress and anxiety in rodents and humans (Herman and Cullinan, 1997). The second study, which involved only social stimuli of positive and negative emotions, showed that OT attenuates the response of the amygdala relative to placebo, regardless of stimulus valence. OT attenuates negative affective evaluations associated with aversively conditioned faces through modulation of the amygdala and fusiform gyrus in non-clinical adults (Petrovic et al., 2008). The participation of OT in the modulation of social behaviour has been strongly supported by the recent discovery of the transmembrane receptor CD38, which seems to be involved in maternal behavior and social recognition by regulation of OT secretion (Jin et al., 2007). These results indicate the need for further studies on OT and anxiety, especially social anxiety.

Epidemiological studies have revealed that the fear of public speaking is highly prevalent among students (Geer, 1965), regardless of gender, ethnic origin or age (Phillips et al., 1997). In addition, public speaking is the most common type of social fear (Furmark et al., 1999; Stein et al., 1994) and is also one of the major symptoms of social phobia (Brunello et al., 2000; Stein et al., 1996). For these reasons, and to obtain a clinical model of anxiety, the need for further studies on OT and anxiety, especially social anxiety.

Materials and methods

Subjects

The study was conducted on 28 healthy male volunteers, who were randomly assigned to receive intranasal administration of OT (Syntocinon®, Novartis, Brazil) or placebo (Syntocinon vehicle®, Novartis). The groups were matched for age, body mass index, personal and parental socioeconomic level, anxiety level and intensity of fear of public speaking, as shown in Table 1. Exclusion criteria were: use of any concomitant medication; history of neurological disease; psychiatric disorders; renal, pulmonary, hepatic or cardiovascular problems; hypertension; smoking; alcoholism; and abusive drug use. Subjects were instructed not to eat or drink (except water) for 2 h before the experiment and not to engage in exercise or ingest alcoholic or caffeinated drinks for a period of 24 h before the experiment. Subjects were blinded to treatment condition until the end of the experiment. All subjects received detailed information about the research and were included in the study after giving written informed consent. The study was approved by the local Ethics Committee.

Screening procedure

The intensity of fear of public speaking was evaluated by the State version of the Self Statement of Public Speaking (SSPS-S) scale (Hofmann and Dibartolo, 2000). To exclude the possibility of harmful use of alcohol, only subjects with a score of less than 3 on the Fast Alcohol Screening Test (FAST) scale were included (Hodgson et al., 2002). To exclude the possible presence of any psychiatric disorder, the short version of the Patient Health Questionnaire (PHQ-2) (Kroenke et al., 2003) and Self-Reporting Questionnaire (SRQ) (Mari and Williams, 1985) scales were used for initial screening, followed by later application of the Portuguese version (Del-Ben et al., 2001) of the structured clinical interview for the DSM-IV (SCID-IV) (First et al., 1997) by a qualified psychiatrist. The socioeconomic class of the subjects and their parents was determined using the Criteria of Socioeconomic Classification for Brazil (CCSEB, 1997).

Oxytocin administration

OT (Syntocinon® Nasal Spray, Novartis) or placebo (Syntocinon® vehicle, Novartis) was administered intranasally in a single dose of 241 U in three applications of 41 U per nostril. This dose was selected based on previous reports demonstrating an anxiolytic effect (Heinrichs et al., 2003; Kosfeld et al., 2005) and the presence of OT in

Table 1. Characteristics of the study groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Oxytocin (n = 14)</th>
<th>Placebo (n = 14)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.8 ± 2.4</td>
<td>23.6 ± 3.1</td>
<td>0.8</td>
</tr>
<tr>
<td>BMI</td>
<td>25.1 ± 4.4</td>
<td>24.2 ± 4.3</td>
<td>0.6</td>
</tr>
<tr>
<td>SSPS-S</td>
<td>38.3 ± 8.1</td>
<td>37.7 ± 4.7</td>
<td>0.8</td>
</tr>
<tr>
<td>BAI</td>
<td>10.5 ± 8.1</td>
<td>10.6 ± 8.7</td>
<td>0.9</td>
</tr>
</tbody>
</table>

BAI: Beck Anxiety Inventory, BMI: body mass index (kg/m²), SSPS-S: State version of the Self Statement of Public Speaking scale
the cerebrospinal fluid after intranasal administration (Born et al., 2002).

**Measures**

**Psychological measures.** The following self-evaluation scales were used for the assessment of subjective states during SPST. (1) The *Visual Analogue Mood Scale* (VAMS) was created by Norris (1971) and translated and validated for Portuguese by Zuardi and Karmiol (1981). The 16 items are grouped into four factors: anxiety, sedation, cognitive impairment, and discomfort (Parente et al., 2005). (2) The *Bodily Symptoms Scale* (BSS) (Zuardi et al., 1993) was used to evaluate somatic symptoms that may indirectly affect anxiety. The scale consists of 21 items on which the subjects indicate how they feel at the time, ranging from not at all (0) to extreme (5). (3) The *State version of the Self-Statements during Public Speaking* (SSPS) scale, used to evaluate fear of public speaking, consists of 10 items that can be divided into positive self-evaluation (items 1, 3, 5, 6 and 9) and negative self-evaluation (items 2, 4, 7, 8 and 10). The subjects must assign scores ranging from 0 (totally disagree) to 5 (totally agree) on each item (Hofmann and Dibartolo, 2000; Osório et al., 2008).

**Physiological and psychophysiological measures.** The following physiological and psychophysiological measurements were assessed: (1) arterial blood pressure, including systolic and diastolic blood pressure, were measured using a mercury sphygmomanometer (Becton Dickinson, Brazil); (2) heart rate was estimated by pulse rate; (3) skin conductance was measured using a computer-controlled, voltage-constant (0.6 V) module with automatic back off (Contact Precision Instruments, UK). Two electrodes (Beckman, UK) were fixed with adhesive tape to the fingers with skin contact made through high conductance gel (KY gel, Johnson and Johnson, Brazil). Both skin conductance (SC) and the number of spontaneous fluctuations in skin conductance (SFSC) were recorded.

**Hormonal measures.** Blood was collected at scheduled times while the subject was seated, and 10 mL from each time point was immediately transferred to polypropylene tubes. Samples used to assay adrenocorticotropic hormone (ACTH) used EDTA as an anticoagulant. Samples used for cortisol measurement were transferred to tubes containing separating gel. All tubes were kept on ice during the experimental session. Blood samples for both ACTH and cortisol assessment were centrifuged at 3000 rpm for 15 min at 4°C, and plasma and serum aliquots were stored in polypropylene tubes at ~20°C until analysis.

**Procedure**

The SPST was conducted as described by McNair et al. (1982) using modifications by Guimarães et al. (1987). The experimental session was performed in a sound-attenuated room with controlled temperature. After a 15-min period of adaptation to the laboratory environment, the first informed consent form was presented for signature, and initial measurements (Baseline) were performed, followed by administration of OT or placebo. At this time, subjects were catheterized for later collection of blood. After 36 min without stimulation, pre-test measurements (Pre-test) were acquired. Immediately thereafter, subjects viewed a videotape containing instructions about the task. Subjects were instructed that they would have 2 min to prepare a 4-min speech about local public transportation. This topic was selected as an emotionally neutral topic requiring common knowledge. Subjects were instructed that they would be interrupted during their talk for the administration of scales and that the speech would be recorded on videotape and later analyzed by a psychologist. At this time, the second informed consent form was presented for signature. If the subject agreed to continue, measurements of the subject’s states during speech preparation (Anticipation) were taken. Next, subjects began to deliver their speech in front of a video camera while viewing their own images on the screen. The speech was interrupted after 2 min so that subjective measurements of performance anxiety (Speech) could be taken. Post-stress measurements were made 15 min (Post-test 1) and 20 min (Post-test 2) after the end of the speech. Each volunteer participated in only one experimental session. Table 2 shows the timeline of the procedures.

**Data analysis**

The results obtained for the 16 items of the VAMS were divided into four factors according to their relative weights: anxiety, sedation, cognitive impairment, and discomfort (Zuardi et al., 1993). The VAMS, SSPS, heart rate, blood pressure (systolic and diastolic), SC and SFSC data were evaluated using a mixed effects linear model for each dependent variable (Gueorguieva and Krystal, 2004) with time and group as fixed effects and subject as a random effect. This procedure is adequate when data are correlated, as each response is influenced by the preceding response. The individual items of the BSS were analyzed by the Kruskal–Wallis test. The possible association between group and social class and group comparisons for discrete variables were analyzed by chi-square tests. SAS software version 9 was used for statistical analysis with the level of significance set at $p < 0.05$.

**Results**

**Psychological measures**

**VAMS.** The effects of oxytocin on VAMS anxiety and sedation factors are shown in Figure 1. Intragroup analyses showed that public speaking was associated with an increase in anxiety in both OT and placebo groups. In the placebo group, anxiety was increased during Anticipation compared with Baseline ($p < 0.05$) and Pre-Test ($p < 0.01$), followed by a decrease at Post-Test 1 ($p < 0.001$) and Post-Test 2 ($p < 0.001$) compared with Anticipation. Anxiety was also higher in the Speech phase compared with Pre-test ($p \leq 0.01$), Post-test 1
In the OT group, anxiety was higher in the Anticipation phase than Pre-test ($p < 0.01$). In addition, there was a significant decrease in anxiety in the Pre-test phase compared with Baseline ($p < 0.01$). Intergroup comparisons at each phase showed that the OT group had a lower level of anxiety than the placebo group at Pre-test ($p < 0.05$), indicating that OT decreased anxiety before, but not during, the public speaking test.

As expected, public speaking reduced sedation. In the placebo group, intragroup comparisons showed that sedation was lower in the Anticipation phase than at Baseline ($p < 0.05$), Pre-test ($p < 0.01$), Post-test 1 ($p < 0.01$) and Post-test 2 ($p < 0.001$). Sedation in the Speech phase was also lower than at Baseline ($p < 0.01$), Pre-test ($p < 0.01$), Post-test 1 ($p < 0.01$) and Post-test 2 ($p < 0.001$). In the OT group, sedation was lower in the Anticipation phase compared with Baseline ($p < 0.05$) and Pre-test ($p < 0.001$). Sedation was also lower in the Speech phase compared with Baseline ($p < 0.05$) and Pre-test ($p < 0.001$). Intergroup comparisons at each phase showed that sedation was higher in the OT group than in the placebo group in the Pre-test (56.7 ± 2.0 mm; 47.5 ± 2.5 mm, $p = 0.003$), Anticipation (47.1 ± 1.7 mm; 39.0 ± 2.9 mm, $p = 0.01$) and Speech phases (47.7 ± 1.3 mm; 41.0 ± 3.0 mm, $p = 0.02$), indicating that OT increased sedation before and during public speaking.
Post-test 2 51.7
Post-test 1 51.0
Speech 54.5
Anticipation 54.1
Pre-test 46.5

and Speech phases (cebo group showed more palpitation (item 11) in the Pre-test Speech and Post-test 1 phases (p < 0.05). In addition, the placebo group showed greater lethargy (item 3) in the Post-test 2 phases (p < 0.05 relative to placebo).

Intragroup and intergroup comparisons showed no statistically significant difference among the various phases and groups regarding the cognitive impairment and discomfort factors of the VAMS, as shown in Table 3.

**BSS.** The OT group showed greater lethargy (item 3) in the Speech and Post-test 1 phases (p < 0.05). In addition, the placebo group showed more palpitation (item 11) in the Pre-test and Speech phases (p < 0.05).

**SSPS.** There were no significant group differences observed during the various phases of positive or negative self-evaluation of public speaking.

**Physiological and psychophysiological measures**

The effect of oxytocin on skin conductance is shown in Figure 2. OT lowered the SC in the Pre-test, Anticipation, Speech and Post-test 2 phases (p < 0.05). No significant differences were detected for the other variables, as shown in Table 4.

**Hormonal measures**

Intergroup comparisons of cortisol and ACTH showed no significant differences between the various phases.

**Discussion**

The present results show that the SPST increased VAMS anxiety in both treatment groups, as revealed by a significant increase of anxiety from the Pre-test to the Anticipation phase, supporting previous findings (Graeff et al., 2003). However, the OT group showed a significant reduction of anxiety from the Baseline to the Pre-test phase, suggesting that OT facilitates habituation of anticipatory anxiety. This hypothesis is supported by the significant difference between OT and placebo groups at the Pre-test phase. This reduction of anxiety during the Pre-test phase compared with Baseline and with placebo was similar to the effect of diazepam, which has also been shown to lower anxiety levels during the Pre-test phase (Zuardi et al., 1993). Neither OT nor diazepam was able to reduce the increase in anxiety generated by public speaking (Anticipation and Speech phases), indicating that this is a different type of anxiety from anticipatory anxiety. In contrast, results have shown that drugs that act primarily on serotinergic neurotransmission do not affect anxiety during the Pre-test phase, but either reduce or increase anxiety in the Anticipation and Speech phases (Graeff et al., 2003). The sedation data support the idea of an anxiolytic action of OT similar to that of the benzodiazepines, which show greater sedation during the Pre-test, Anticipation and Speech phases in the OT group relative to controls. Diazepam and oxytocin have been shown to exert anxiolytic effects in the same experimental paradigms. Recently, an electrophysiology study showed that oxytocin can facilitate the effects of diazepam in the amygdala (Viviane et al., 2010).

McNaughton and Corr (2004) have proposed that the same set of longitudinally organized brain structures, located in the midbrain and frontal cortex, govern anxiety and fear. They suggest that, while both midbrain and forebrain areas are involved in anxiety and fear, the more rostral areas, such as...
as the prefrontal cortex and the amygdala, would predominantly control anxiety, whereas the more caudal structures, such as the hypothalamus and midbrain periaqueductal gray matter, would predominantly control fear and panic. Defensive distance is likely to be a key factor, since a functional neuroimaging study in humans has shown that neural activation shifts from the prefrontal cortex to the periaqueductal gray matter as threat becomes more imminent (Mobb et al., 2007). Thus, based on the responses observed to different types of drugs, it may be assumed that in the SPST anticipatory anxiety occurs from the beginning of the pre-test phase, and fear of public speaking occurs in the speech preparation/anticipation and performance phases (Graeff et al., 2003). Among the brain structures involved in defense, OT receptors have been detected in the amygdala (Condes-Lara et al., 1994; Kremarik et al., 1993; Mantella et al., 2003), septum (Insel et al., 1993; McCarthy et al., 1996; Mantella et al., 2003), hippocampus, and hypothalamus (Blume et al., 2008; Mantella et al., 2003; Sofroniew, 1983). In particular, a significant activation of the oxytocinergic system in the central amygdala in response to swim stress suggests that OT receptor-mediated mechanisms within the amygdala are involved in the generation of passive stress-coping strategies (Ebner et al., 2005). These findings were partially confirmed in humans in an fMRI study, which showed that OT modulates the expression of evaluative conditioning for socially relevant faces via influences on amygdala and fusiform gyrus, which may explain the prosocial effects of OT (Petrovic et al., 2008). With the exception of the hypothalamus, where oxytocin exerts hormonal functions and has anxiolytic effects mediated by local OT receptor-induced activation of the ERK 1/2 cascade (Blume et al., 2008), rostral structures are primarily involved in anxiety rather than fear (McNaughton and Corr, 2004). In contrast, OT receptors in the midbrain periaqueductal gray matter (Ogawa et al., 1992) are likely involved in fear, as this structure is thought to be activated in panic attacks (Graeff, 2004).

The present results show that oxytocin failed to reduce fear of public speaking, the most feared social situation. This was unexpected in view of previously reported evidence suggesting that OT promotes social facilitation (Insel and Huihian, 1995; Williams et al., 1994). For instance, Heinrichs et al. (2003) found anxiolytic effects of intranasal oxytocin (24IU) using the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993), which primarily consists of a public speaking task and mental arithmetic performed in front of an audience. The authors also observed that the combination of oxytocin and social support exhibited the lowest cortisol concentrations, as well as increased calmness and decreased anxiety during stress. Nevertheless, it is possible that doses above the range used in the current study could be effective. Higher doses should therefore be investigated, especially in patients with social anxiety disorder.

An anxiolytic action of OT is supported by the present study, showing lower results for SC in the OT group during the Pre-test, Anticipation, Speech and Post-test 2 phases. Fowles (1980, 2000) suggested that SC is involved in anticipatory anxiety, whereas changes in SFSC and heart rate, which were not affected by oxytocin in the current study, are more related to fear. Neuroimaging studies have shown that SC and SFSC are regulated by different neural systems. Specifically, the medial prefrontal cortex is involved in SC (Nagai et al., 2004), which involves anticipatory anxiety about future punishment (Bechara et al., 1999).

In the present study, no significant increases in plasma cortisol or ACTH were observed during any phase of the experimental session. This finding agrees with recent reports of salivary cortisol levels under very similar experimental conditions, indicating that the SPST does not activate the hypothalamic–pituitary–adrenal axis (Garcia-Leal et al., 2005). Nevertheless, there are contradictory results in the literature, as some authors report no change in serum cortisol levels (Becker et al., 1996; Lupien et al., 1997) while others report increased cortisol following procedures similar to the SPST (Buchanan et al., 1999; Nicolson and Van Diest, 2000).

In summary, the present results and previous reports in experimental animals (Bale et al., 2001; McCarthy et al., 1996; Mantella et al., 2003, 2005; Neumann et al., 2000; Windle et al., 1997) and in humans (Heinrichs et al., 2003) suggest that OT has anxiolytic properties.

**Conclusions**

The present study suggests that OT reduces anticipatory anxiety and the related psychophysiological measure of SC in healthy volunteers performing public speaking. In contrast, OT failed to affect anxiety during public speaking,

---

**Table 4. Physiological and psychophysiological changes induced by the Simulated Public Speaking Test**

<table>
<thead>
<tr>
<th>Phase</th>
<th>Heart Rate</th>
<th>Systolic Blood Pressure</th>
<th>Diastolic Blood Pressure</th>
<th>Spontaneous Fluctuations (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OT (mmHg)</td>
<td>PL (mmHg)</td>
<td>OT (mmHg)</td>
<td>PL (mmHg)</td>
</tr>
<tr>
<td>Baseline</td>
<td>71.4 ± 2.7</td>
<td>73.6 ± 2.2</td>
<td>116.7 ± 2.8</td>
<td>116.4 ± 2.3</td>
</tr>
<tr>
<td>Pre-test</td>
<td>66.4 ± 1.8</td>
<td>69.9 ± 1.7</td>
<td>117.3 ± 2.5</td>
<td>113.7 ± 2.1</td>
</tr>
<tr>
<td>Anticipation</td>
<td>69.9 ± 2.0</td>
<td>74.9 ± 2.5</td>
<td>121.1 ± 3.1</td>
<td>118.6 ± 1.6</td>
</tr>
<tr>
<td>Speech</td>
<td>64.3 ± 1.9</td>
<td>68.4 ± 2.1</td>
<td>118.6 ± 3.2</td>
<td>118.6 ± 2.1</td>
</tr>
<tr>
<td>Post-test 1</td>
<td>63.1 ± 1.6</td>
<td>68.5 ± 2.1</td>
<td>115.4 ± 2.7</td>
<td>114.3 ± 2.3</td>
</tr>
<tr>
<td>Post-test 2</td>
<td>63.9 ± 1.6</td>
<td>68.5 ± 2.0</td>
<td>115.1 ± 3.0</td>
<td>114.1 ± 1.8</td>
</tr>
</tbody>
</table>

Data are reported as means ± SEM. OT: oxytocin, PL: placebo
cardiovascular changes, or SFSC. Therefore, OT seems to exert an anxiolytic action similar to that of benzodiazepines.

Acknowledgements

We thank Sandra Bernardo for assistance with data collection and analysis.

Funding

This work was supported by a grant from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; 2002/13197-2). FGG is the recipient of research fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and Fundação de Apoio ao Ensino, Pesquisa e Assistência (FAEPA) do Hospital das Clínicas da FMERPUSP. JASC and AWZ are recipients of research fellowships from CNPq (Brazil). DIC is recipient of a Capes fellowship.

Conflict of interest

None declared.

References


