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Phenelzine treatment increases transcription factor AP-2 levels in rat brain

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Abstract

Background: The elevations of noradrenaline (NA) and serotonin (5-HT) levels in response to acute serotonin reuptake inhibitor (SSRI) or tricyclic antidepressant (TCA) exposure are not consistent with the time course for the therapeutic action of these antidepressants. Thus, neuronal adaptations are needed for the therapeutic effect to arise. Transcription factor Activating Protein-2 (AP-2) is critical for mammalian neural gene expression. Several genes involved in brainstem CNS transmitter systems, especially the monoamines, have AP-2 binding sites in their regulatory regions. We have previously shown that treatment with citalopram and imipramin resulted in a decrease in AP-2 α and AP-2 β levels in rat brain. We have also reported an association between a specific genotype of AP-2 β to personality traits, binge-eating disorder and platelet monoamine oxidase (MAO) activity.

Results: Subchronic administration (10 days) of phenelzine (PLZ) increased the levels of AP-2 α , AP-2 β and the DNA binding activity of AP-2 in nuclear extracts prepared from rat whole brain when compared with sham treated animals.

Conclusion: These data suggest that AP-2 is not involved in the therapeutic effect of antidepressants. Rather, the effects of antidepressants seen on the levels of AP-2 might be involved in the expression of side-effects during the lag-period.

Background

It has been suggested that the therapeutic action of antidepressant drugs, such as TCAs and SSRIs, are directly related to their uptake-blocking capability. However, since the onset in therapeutic action of these drugs usually is delayed for several weeks, the hypothesis, that the rapid, acute actions of these drugs in blocking NA and/or 5-HT reuptake are responsible for the long-term clinical antidepressant effect has been questioned, suggesting that their

mechanism of action involves neuronal adaptations [1,2]. Several studies have shown that antidepressant treatment has effects on transcription factors, which might be responsible for the neuronal adaptations that are needed for the therapeutic effect to arise [3–5].

AP-2 is a cell type-specific DNA-binding transcription factor family of different, yet closely related, proteins with a molecular weight around 50 kDa. Four different AP-2

genes, i.e., AP-2 α , AP-2 β , AP-2 γ , and AP-2 δ have been identified [6–9]. AP-2 is one of the critical factors for neural gene expression in mammals [10]. Several of the genes involved in brainstem CNS transmitter systems, of fundamental importance for behavior, have multiple AP-2 binding sites in their regulatory regions, see [4] and references therein. AP-2 binds to GC-rich sequences in the genome [11]. The cis-acting DNA sequences 5'-(G/C)CCCA(G/C)(G/C)(G/C)-3' and the palindromic sequence 5'-GCCNNNGGC-3' are considered as consensus AP-2 binding sites for all AP-2 proteins [12].

AP-2 seems to mediate transcriptional activation in response to both the phorbol-ester- and diacylglycerol-activated protein kinase C, and the cAMP-dependent protein kinase A pathway [7,13].

We have earlier reported that AP-2 β genotype is associated with certain personality traits as estimated by the Karolinska Scales of Personality (KSP) [14], platelet MAO activity [15] and binge-eating disorder [16]. However, AP-2 β genotype was not associated with schizophrenia [17]. Moreover, brainstem levels of AP-2 α and AP-2 β were found to be positively correlated to forebrain monoamine levels in adult rats, e.g. 5-HT turnover and NA-levels in frontal cortex [18]. We have previously shown that subchronic treatment with citalopram (CIT) and imipramin (IMN) resulted in a decrease in AP-2 α and AP-2 β levels and DNA-binding activity of AP-2 in rat whole brain [4]. In the present study, we report that subchronic treatment with the non-selective MAO-inhibitor (MAO-I) PLZ resulted in an increase in both AP-2 α and AP-2 β levels and DNA-binding activity of AP-2.

Results

Subchronic administration (10 days) of PLZ significantly increased the DNA binding activity of AP-2 in nuclear extracts prepared from rat whole brain when compared with sham treated animals (Fig. 1), i.e. 1.57 ± 0.095 (Range: 1.45–1.60), compared with 0.688 ± 0.204 (Range: 0.38–0.91) (mean relative optical density \pm SD, $p < 0.0001$). Representative EMSA gels for PLZ and Sham treated animals are shown in figure 2 (Fig. 2). One single band was seen on EMSA gels after an overnight exposure, which supposedly represents AP-2 binding to the labelled probe. By including different concentrations of cold AP-2 probe in the binding reaction, AP-2 binding to the labelled probe was reduced with increased concentration of cold AP-2 probe. A 50 fold excess of a non-labelled AP-2 probe almost completely blocked binding to labelled probe. In contrast, when a 50 fold excess of a non-labelled mutated AP-2 probe or a non-labelled AP-1 probe was included in the binding reaction, AP-2 binding was not affected (Fig. 3)

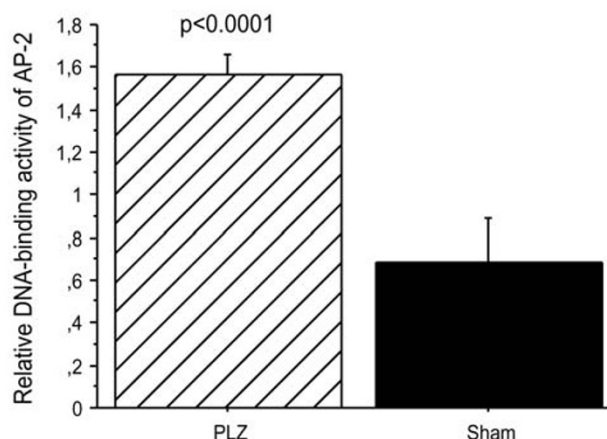


Figure 1

Treatment with PLZ (10 days) increases the DNA-binding activity of AP-2 in rat brain. Relative DNA binding activity of AP-2 was determined by EMSA analyses on nuclear extracts prepared from rat brain. PLZ treated animals were compared with a sham treated control group. The results were considered statistically significant when $p < 0.05$.

When analysing the level of AP-2 α , there was a tendency that AP-2 α was increased in the PLZ treated rats, i.e. 7.43 ± 2.88 (Range: 4.94–10.48) compared with 4.11 ± 2.03 (Range: 1.61–6.26) (mean relative OD \pm SD, $p = 0.068$) (Fig. 4a). The level of AP-2 β protein was increased in the PLZ treated rats in comparison to sham treated animals, i.e. 5.86 ± 0.63 (Range: 5.13–6.80) compared with 4.30 ± 0.62 (Range: 3.51–4.92) (mean relative OD \pm SD, $p = 0.0043$) (Fig. 4b).

Discussion

Several research groups are currently discussing the possibility that serotonergic gene transcriptional control regions, or transcription factors themselves, could be future targets for antidepressant drugs [16,19–22].

We have previously shown an effect of treatment with CIT and IMN on AP-2 in rat brain. Both drugs decreased the levels of AP-2 α , AP-2 β and DNA-binding activity of AP-2 after 10 days of treatment [4]. The present data regarding PLZ, an unselective irreversible inhibitor of MAO-A and MAO-B, goes in the opposite direction. The reason for this could be explained in several ways. Blier and de Montigny (1998) suggested that long-term administration of MAO-Is had different effects on both terminal autoreceptor- and postsynaptic responsiveness than SSRIs [23]. These different effects on receptor responsiveness would explain differences in downstream effects on AP-2 after treatment with MAO-I and SSRIs.

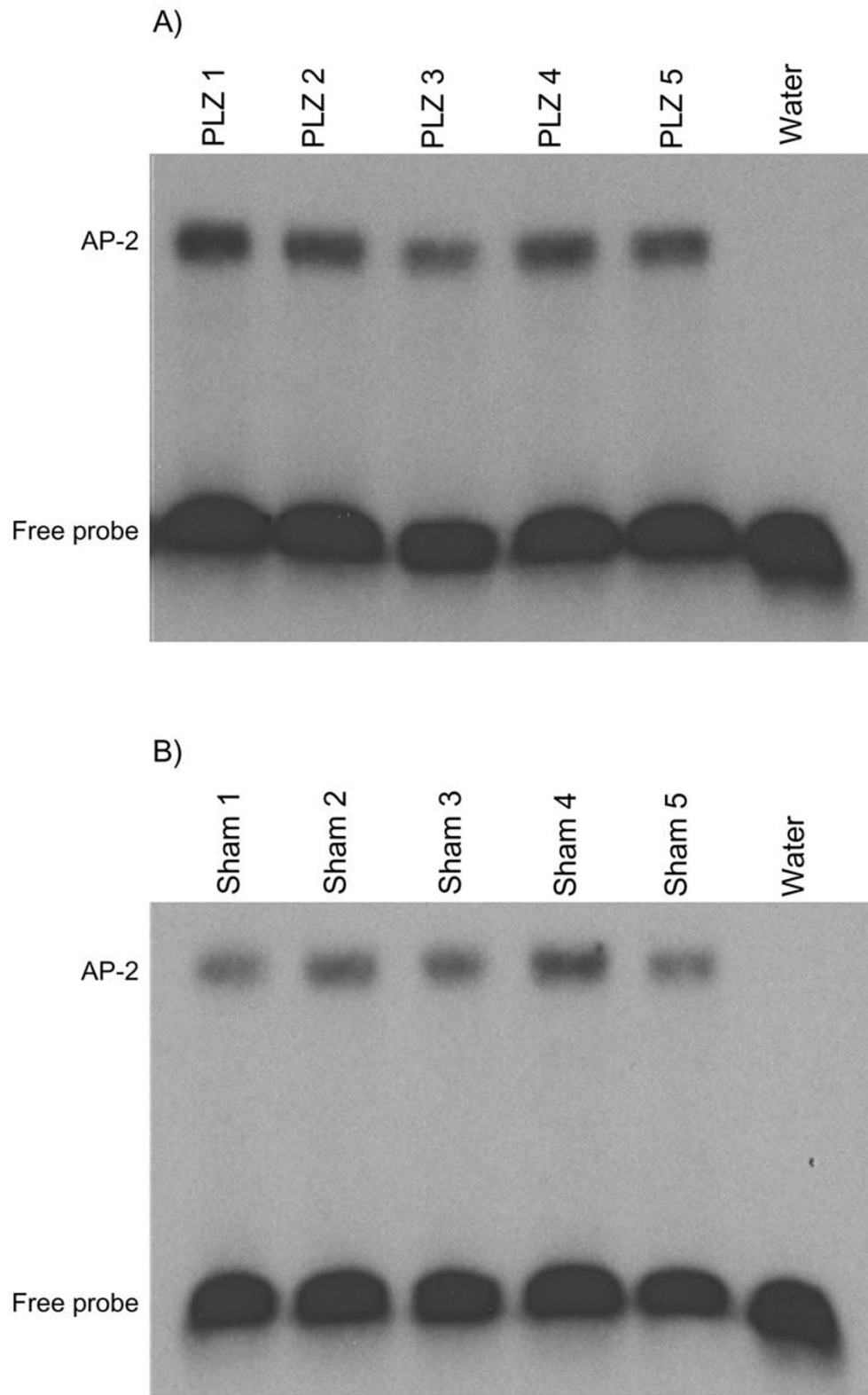


Figure 2
Representative EMSA autoradiograms showing A) PLZ and B) sham treated animals. Each group consisted of five rats and each rat was analysed twice for accuracy.

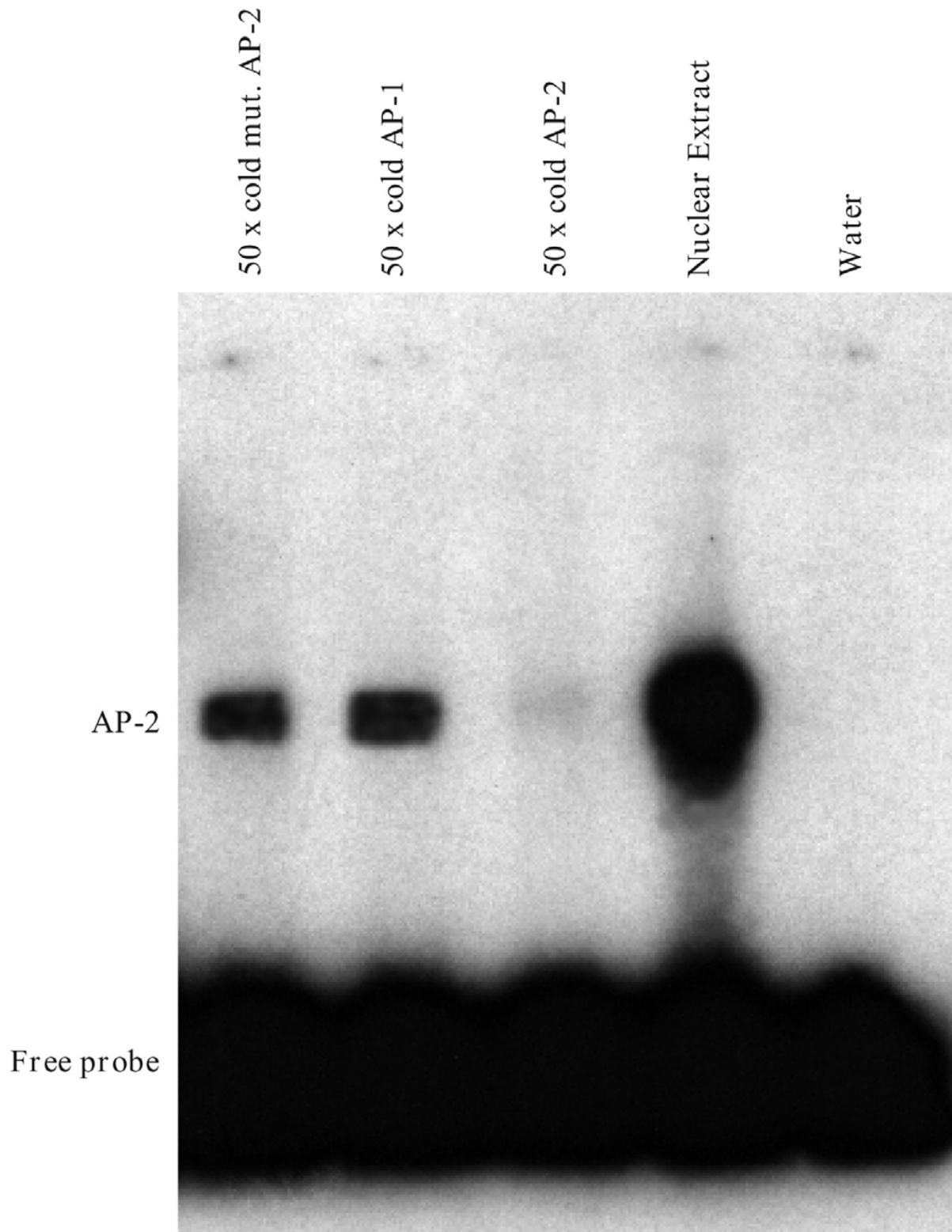


Figure 3
A representative EMSA autoradiogram showing the specificity of binding to the labelled AP-2 probe is shown. Arrows indicate the main band considered as AP-2 binding to the labelled probe, free probe is also indicated.

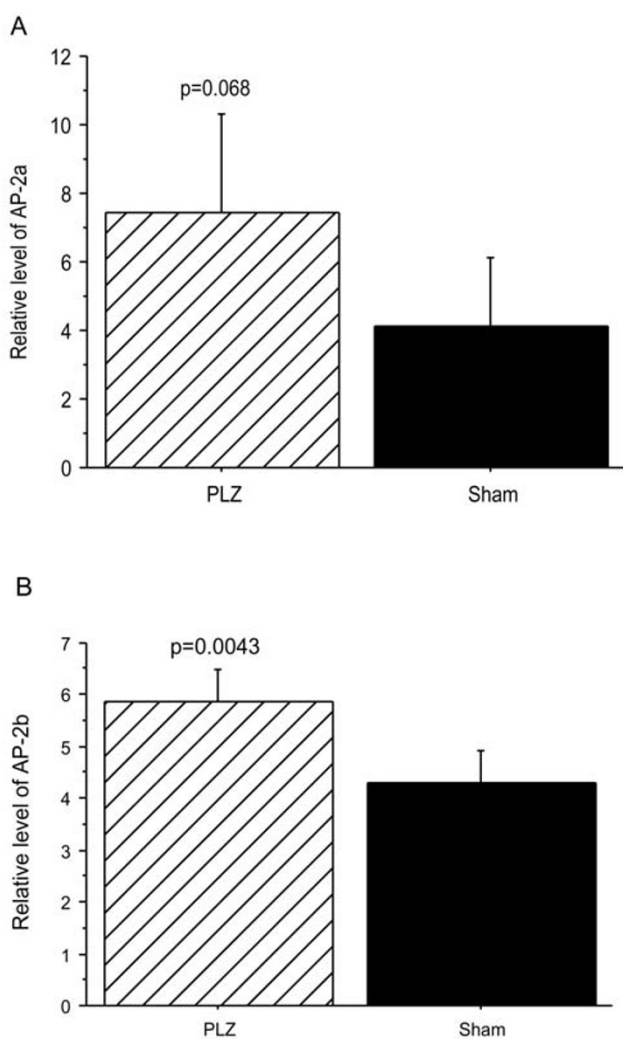


Figure 4
Relative levels of AP-2 α and AP-2 β were determined by ELISA analyses after 10 days of PLZ treatment. A) There is a tendency that PLZ treatment increases levels of AP-2 α in rat brain. B) Treatment with PLZ increases levels of AP-2 β in rat brain. The results were considered statistically significant when $p < 0.05$.

It has been shown that treatment with PLZ displays an increase in the levels of brain monoamines after one week, but that levels of NA, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) return to baseline levels after two weeks, even though MAO activity remained significantly inhibited at these times [24]. We have previously shown that CIT treated rats displayed significantly lower levels of AP-2 α and AP-2 β and DNA-binding activity of AP-2 after 7 days of treatment, and that those levels were normalised after 21 days [5]. This is in line with the fact

that we, in another study, have found positive correlations between brainstem levels of AP-2 α and AP-2 β and 5-HT turnover and levels of NA in the forebrain of rats [18]. The lag period observed before therapeutic onset with antidepressants is associated with several side-effects, and even an increased risk for suicide. One may speculate that the down-regulation of AP-2 seen after treatment with CIT and IMN, as well as the up-regulation seen after PLZ treatment, are not involved in the therapeutic effect of these antidepressants. Rather, the effects of antidepressants seen on the levels of AP-2 might be involved in the expression of side-effects during the lag-period.

We have previously reported an association between AP-2 β genotype and platelet monoamine oxidase B activity [15]. Thus, it seems as if transcription factor AP-2 is involved in the regulation of MAO-B gene expression. One way to explain the increase in AP-2 levels and DNA-binding activity after PLZ treatment would be that when blocking MAO-B with PLZ there is an up-regulation of AP-2 in order to compensate for the decreasing amount of MAO-B enzyme.

Conclusions

An increased knowledge of the molecular mechanisms involved in the initial lag-period before the therapeutic effect of antidepressant treatment, might make it possible to generate compounds with a faster therapeutic onset and less side-effects. The use of a new class of compounds directly affecting transcription factor mechanisms is currently discussed [16,19–22].

Methods

Adult, male Sprague-Dawley rats (10 weeks of age, B-K Universal AB, Sollentuna, Sweden) were housed in groups of five with food and water ad libitum. Animals were administered PLZ (10 mg/kg, KEBO, Stockholm, Sweden) subcutaneously with daily injections for 10 days. PLZ was dissolved in saline (NaCl, 9 mg/ml, Pharmacia – Upjohn, Uppsala, Sweden). Sham treated animals received saline injections of the same volume as that given for drug treatments. The animals were sacrificed 24 hours after the final injection. After sacrifice the cerebrum was dissected and the right hemispheres were prepared for electrophoretic mobility shift assay (EMSA) and Enzyme-Linked Immunosorbent Assay (ELISA) analyses. The study was approved by the local ethics committee.

Nuclear proteins were extracted essentially according to the protocol by Dignam and co-workers [25]. For the EMSA analyses the AP-2 consensus dsDNA oligos (5'-GATCGAAGTACCGCCCGCGGCCCGT-3') were 5'-end labelled with T4 polynucleotide kinase according to the manufacturer (United States Biochemical, Cleveland,

Ohio). Typically, the labelled probes had a specific activity of $\sim 2 \times 10^5$ cpm/pmol DNA.

The EMSA binding reaction was carried out as previously described [5]. The DNA-binding activity of AP-2 was analyzed in relation to each of the different treatment groups. Each treatment group consisted of five rats and each rat was analysed twice for accuracy. The dsDNA probe used in the EMSA analyses in order to analyse the DNA binding activity of AP-2 has been used in many other studies of AP-2 and it has been confirmed that AP-2 binds to the probe by interacting with the AP-2 consensus site [4].

The ELISA was performed as previously described [5]. The plates were analysed in an ELISA reader (Molecular Devices, Thermo Max) at optical density (OD) 405/490. The OD of the AP-2 isoforms for each rat was correlated to a value in a standard curve, where known concentrations of antibody are plotted against optical density. The value from the standard curve was then divided with the concentration of the total protein in the nuclear extracts. The quota was used as a relative amount of AP-2 α and AP-2 β . Each treatment group consisted of five rats and each rat was analysed twice for accuracy.

The statistical comparisons between PLZ treated rats and controls were done by the use of Student's t-test. All calculations were performed using StatView 5.0 software (SAS Institute Inc., Cary, NC, USA). Results have been considered statistically significant when $p < 0.05$.

Author contributions

MD planned the experiments, carried out the EMSA and ELISA analyses, drafted the manuscript and performed the statistical analyses. CB participated in the ELISA analyses. LO conceived of the study and participated in the design and coordination of the study. All authors read and approved the final manuscript.

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