PROBABLE ROLE OF GUANIDINE COMPOUNDS IN IMMUNODINSFUNCTION OBSERVED IN CRONIC RENAL FAILURE

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Chronic renal failure (CRF) has been usually associated with a significant reduction in serum L-arginine (L-ARG) levels, reduced nitric oxide (NO) synthesis and with accumulation of some nitrogen compounds deriving from protein catabolism like creatine (CRT), creatinine (CRTN), guanidine (GND) and methylguanidine (MG; 1,2). It has been proposed that nitrogen compounds accumulation could be responsible of some manifestations of uremic syndrome (3-6).
Changes in monocyte functions have been recognised as one of the most important key factor responsible for the immunological disorders associated with uraemia (5) and it has been demonstrated that high blood concentrations of nitrogen compounds, as MG, could be responsible of both immunodisfunctions and uremic syndromes (4). Although the mechanism for the development of these disorders are still unclear we have previously demonstrated that the uremic toxins, MG and GND, inhibited *in vitro* nitric oxide (NO) production via constitutive (cNOS) and inducible (iNOS) isoforms of NO synthase (4) and TNF-α levels in J774.A1 macrophages and, *in vivo*, in LPS-treated rats (7).
Nitric oxide production by iNOS is a crucial step both in the activation of immunoresponsive cells and in the mechanism of citotoxicity NO mediated. The aim of this study is to evaluate if some uremic toxins like CRT, CRTN, GND, MG could interfere in macrophagic activity modulating iNOS activity and expression.
Expression of iNOS was induced by *E. Coli lipopolysaccharide* (LPS) in murine macrophage J774.A1 cell line. Tested compounds were added 24 h after LPS ($6x10^3$u/ml) and the incubation continued for further 24 h. Nitric oxide release was evaluated in cell medium as nitrite (NO_2^-) by Griess reaction (8).
The NO release, by J774.A1 macrophages treated with LPS, was 7.8±2.4 µM NO_2^-. The addition of CRT or CRTN did not produced any significant variation on NO release. In contrast, treatment with MG (0.1, 1 and 10 mM) or GND (0.1, 1 and 10 mM) significantly inhibited NO release by 9.64±14.0%, 43.3±5.4% and 93.3±3.4% for MG ($P<0.001$) and 10.2±14.0%, 34.1±9.7% and 80.8±9.2% for GND ($P<0.001$) respectively. Their inhibitory effects were reverted by adding, to the culture medium, L-ARG (10 mM) supplementation. These results show that both MG and GND are able to inhibit, as false substrate, NO production.
The next step was performed in order to verify any effect of guanidine compounds on the expression of iNOS. Here we report, as preliminary results, the effect of MG-pretreatment on iNOS expression. J774.A1 macrophages were pre-exposed to MG (0.1-10 mM) for 18 h or 30 min. before or 24 h after LPS challenge. Protein expression was detected on cell lysates by Western blot analysis 24 h after LPS challenge, using a monoclonal antibody for murine iNOS and NO was detected as previously indicated (8).
Analysis by Western blotting revealed a significant ($P<0.01$) and concentration related inhibition of iNOS expression in cells pretreated with MG either 18 h or 30 min before LPS, but not when it was added 24 h after LPS challenge (Fig. 1).

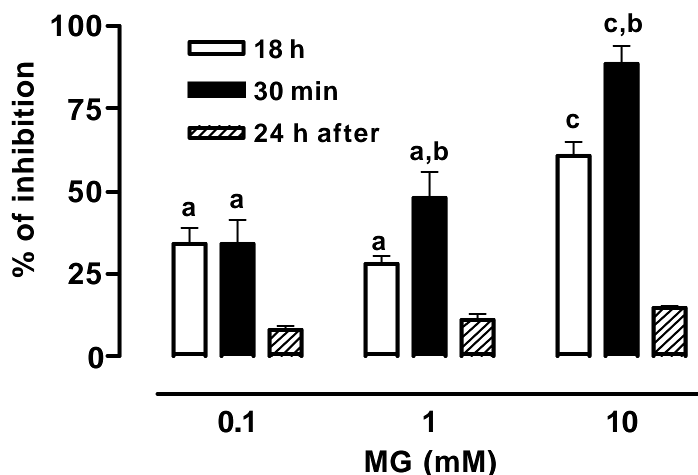

Fig. 1 Effect of MG, added 18 h or 30 min before or 24 h after LPS challenge, on iNOS expression in J774.A1 macrophages.
a $P<0.01$ vs. 24 h
b $P<0.005$ vs. 18 h
c $P<0.001$ vs. 24 h

In these conditions NO levels were markedly and significantly inhibited ($P<0.001$) adding MG either before or after LPS challenge.
Our results show that guanidine compounds, like MG and GND, interfere with NO pathway through the inhibition of iNOS activity. Furthermore, MG interfered with NO pathway also inhibiting the expression of iNOS. The latter effect could be related to probable action of MG on transcriptional mechanisms which regulate protein expression. Indeed, this hypothesis is validate by the fact that MG reduces significantly, both *in vitro* and *in vivo*, TNF-α release, as we have previously reported (7).
These findings could have significant implications in unravelling the complex mechanism involved in uremic patient immunodeficiency were an increasing of these uremic toxins is observed. Furthermore, these effects of guanidine compounds could be increased by the impaired L-ARG concentration, which occurs in CRF. However, in our experimental model these uremic toxins have been tested individually, and we can not exclude the possibility of a synergistic action in uremic condition, where all these products accumulates together.

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