Use of neostigmine - atropine plus antivenom in the experimental envenomation by *Micrurus* venom. Preliminary results.

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ABSTRACT

Venoms of most elapids are neurotoxic being their most important components alpha-neurotoxins and PLA_2s. The treatment with neostigmine and atropine has been suggested to revert the toxicity of nicotinic toxins. The usefulness of an alternative tool is very important due to the lack of antivenom for some Elapids like *Micrurus* (*M.*), due the scarcity of specific antivenoms (AV). We assayed in rescue experiments (mice challenged with mortal doses) the usefulness of the combination neostigmine–atropine (NA) alone or combined with AV on the venoms of *Naja (N.) kaouthia, M. altirostris, M. pyrrhocryptus and M. surinamensis*. The Antivenom used were therapeutic anti-*Micrurus* and experimental anti-*Naja siamensis* antivenoms. Despite that all the cases received a single dose of 20 µg atropine + 2.5µg neostigmine by i.p. route delayed the time of death ($p<0.05$), no good protection was observed using only this treatment. In the other hand, only high doses of AV achieve some level of protection. Nevertheless the combination of NA plus AV, reduced the mortality, as well as the dose of antivenom required for protection in all the cases regarding these treatments used alone. In the case of *M. altirostris* venom, the protection using NA was from 0 to 20% and that using 50 µl of AV ranged from 0 to 60%, while using the combined treatment the protection was from 80 to 100% (p 0.046 and 0,02 regarding AV or NA alone). In the other cases an improvement was observed regarding the uses of NA alone, AV alone (250 µl) or their combination. In the case of *N. kaoutia*= 0%, 20%, 40% respectively, *M. pyrrhocryptus*= 0%, 60%, 100% and *M. surinamensis*= 0%, 0 to 20%, 40-80%. These preliminary results suggest the utility of this combination for the treatment of these envenomations, which could be helpful to reduce the dose of AV.

INTRODUCTION

Background: most important components in elapid venoms are α-neurotoxins that block the acetylcholine receptor at nicotinic level and β-neurotoxins that act pre-synapsis level. Coral snakes possess both. By this reason the use of acetylcholine inhibitors and atropine was suggested as a therapeutic tool to treat these envenomations, as it is used for several species of Asiatic elapids in which α-neurotoxins are the main components. This was studied *in vitro* in several *Micrurus* venoms and showed to be useful only in case of absence of presynaptic toxins. Nevertheless, the few cases of human envenomation treated with acetyl-cholinesterase inhibitors were caused by *Micrurus frontalis*. There is no information available on the usefulness of this therapeutic tool in other *Micrurus* envenomations and the clinical information indicates that postsynaptic nicotinic blockade by venom toxins does not imply their reversal by anticholinesterases, suggesting caution in extrapolating the beneficial effects *in vitro* to clinical envenoming. These observations were done on clinical cases or experiments related with *M. frontalis*, which venom have α-neurotoxins as major component. We observed that acetyl-cholinesterase inhibitors + atropine in a single dose does not protect mice challenged with *Micrurus* venoms, but drastically reduce the necessary dose of antivenom required to rescue mice challenged over LD_{100} doses of *Micrurus* or *Naja* venoms. By this reason we investigate *in vivo* usefulness of the combination of neostigmine +atropine, and its combination with antivenoms on several coral snake venoms from different regions of America and on some *Naja* venoms.

MATERIAL AND METHODS

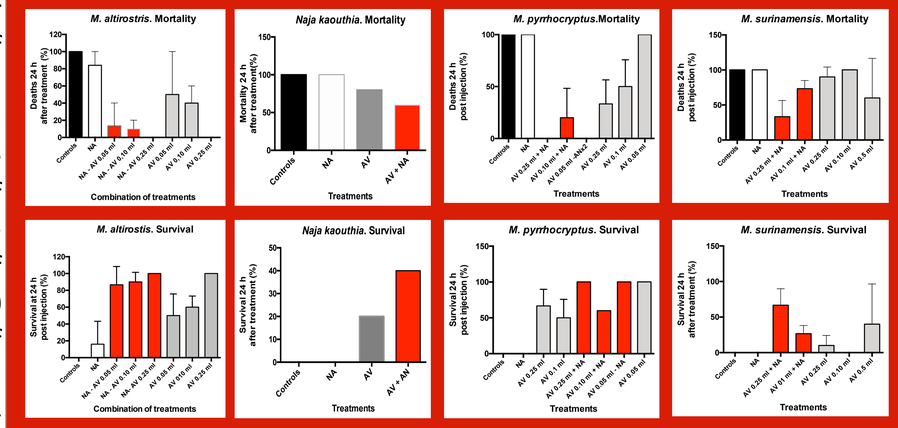
We assayed in rescue experiments (mice challenged with mortal doses) the usefulness of the combination neostigmine–atropine (NA) alone or combined with AV. The venoms of *Naja (N.) kaouthia* (from the bank of venoms of our lab, venom obtainded from a confiscated specimen) , *M. altirostris* (pool from Misiones, Argentina), *M. pyrrhocryptus* (pool from Argentina) and *M. surinamensis* (Letizia, Colombia). **Figure 1**. The challenge doses were 1.2 MMD by the subcutaneous route. The time of death was established in all the cases. The treatment with neostigmine – atropine was determined after several tested doses and times of application after venom injection. The treatment applied was a dose of 20 µg atropine + 2.5 µg neostigmine by i.p. route (NA) delayed the time of death ($p<0.05$), 15 minutes after venom injection. The antivenoms used were therapeutic anti-*Micrurus antivenom* (INPB) and experimental anti-*Naja siamensis* antivenoms by the i.p. route. Several doses were tested. These when applied, were used 15 minutes after the venom injection

FIGURE 1.
From left to right: *M. pyrrhocryptus* (Santiago del Estero, Argentina), *M. surinamensis* (Letizia, Colombia), *M. altrostris* (Misiones, Argentina) and *Naja kaouthia* (confiscated, in captivity).

RESULTS

The percentages of mortality and survival of the combinations of treatments with in the different cases are expressed in **Figures 2.a, 3.a**, **4.a** and **5.a** (mortality) **and 2.b, 3.b, 4.b** and **5.b** (survival). In the case of *M. altirostris* venom (**Figure 2**) , the protection using NA was from 0 to 20% and that using 50 µl of AV ranged from 0 to 60%, while using the combined treatment the protection was from 80 to 100% (p 0.046 and 0,02 regarding AV or NA alone). In the other cases an improvement was observed regarding the uses of NA alone, AV alone (250 µl) or their combination. In the case of *N. kaoutia*= 0%, 20%, 40% respectively (**Figure 3**), *M. pyrrhocryptus*= 0%, 60%, 100% (**Figure 4**) and *M. surinamensis*= 0%, 0 to 20%, 40-80% (**Figure 5**).

Figure 2 **Figure 3** **Figure 4** **Figure 5**



Figures 2 to 5. Mortality and survival at 24 h post injection of venom alone (Control, 1.2 MMD), neostigmine + atropine (NA) alone, or mixing with antivenom (AV). The venom was injected by the subcutaneous route while the treatment through the intraperitoneal route. Bars expressed the mean and deviation bars the standard deviations.

DISCUSSION

- In all the cases in which mice received a single dose of atropine + neostigmine, the time of death was prolonged ($p<0.05$), but no good protection was observed using only this treatment since after some time, mice dead in almost all the cases. Antivenom alone only in some ocassions, provide some protection. Nevertheless the combination of NA plus AV, reduced the mortality, as well as the dose of antivenom required for protection in all the cases.
- These preliminary results suggest the utility of this combination for the treatment of these envenomations, which could be helpful to reduce the dose of AV.
- Other schemes considering repeated doses or the change of acetilcholinesterase inhibitors, coud provide more information on the usefulness of the combination alone or combined with antivenoms or respiratory assistance in order to treat this experimental envenomation.
- The combination of this therapy with antivenom in addition to antagonize the action of alpha-neurotoxins, could help to reduce the toxicity of other components of the venom, like phospholipases.
- The known of the real utility of this combination could be of help in the treatment of this type of envenoming due its complicated mechanism of envenoming and the ussualy reduced stock of this type of antivenoms in the health centers.

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