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Long-term behavioral consequences of soman poisoning in mice

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Abstract

We investigated the long-term (up to 90 days) consequences of soman intoxication in mice on weight, motor performances (grip strength, rotarod) and mnemonic cognitive processes (T-maze, Morris water maze test). First, a relative weight loss of 20%, measured 3 days after intoxication, was evidenced as a threshold beyond which neuropathological damage was observed in the hippocampus. Animals were then distributed into either low weight loss (LWL) or high weight loss (HWL) groups according to the relative 20% weight loss threshold. Compared to controls, both groups of poisoned mice quickly exhibited a decrease in their motor performance subsequent to an acute soman toxicity phase. Then, total motor recovery occurred for the LWL group. Comparatively, HWL mice showed only transient recovery prior to a second decrease phase due to soman-induced delayed toxicity. One month after intoxication, mnemonic cognitive performances of the LWL group were similar to controls while the HWL group did not exhibit any learning skill. Three months after poisoning, compared to controls, the LWL group showed similar mnemonic performances in the maze test but a mild deficit in the Morris water maze task. At the same time, learning skills slightly recovered in the HWL group. Mnemonic cognitive data are discussed in relation to the neuropathology, neurogenesis and sprouting occurring in the hippocampus of soman-intoxicated animals.

Keywords: Soman; Spatial learning and memory; Rotarod; Grip strength; Neuropathology

1. Introduction

Organophosphates, such as the powerful warfare neurotoxicant soman (pinacolyl methylphosphono-fluoridate), are irreversible cholinesterase inhibitors. Acute administration of soman rapidly induces the development of toxic signs including hypersecretion, respiratory distress, cardiovascular dysfunction, muscular convulsions and seizure activity (Lemercier et al., 1983; McDonough et al., 1998; Shih, 1993). Epileptic status severity can then promote irreversible brain damage. The minimal duration of seizure activity necessary for irreversible damage is about 20 min and the damage process accelerates greatly after this minimal time has elapsed (McDonough et al., 1995).

To date, the neuropathological consequences of soman intoxication have been mainly studied over a rather brief period of time, up to 30–40 days after poisoning (Lemercier et al., 1983; McDonough et al., 1998; Shih, 1993). However, we recently reported that, when studied over a longer period of time, soman poisoning induced a two-phase neurodegenerative process accompanied by delayed neuronal regeneration (Collombet et al., 2006). Thus, in the hippocampal CA1 field in mice, an acute toxicity phase was observed on post-soman day 1, leading to the rapid death of about 20% of the neurons. At the same time, a massive quantity of degenerating neurons (about 50% of the total number of CA1 pyramidal neurons) appeared in the CA1 field and survived for 2 months post-exposure. Then, on post-soman day 60, a delayed toxicity phase was initiated by slow death of degenerating neurons, leading to

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complete depletion of these neurons on post-soman day 90. While this two-phase degenerative process occurred, neuronal regeneration initiated 1 month after exposure was also detected in the CA1 field. Then, the number of new neurons represented about 20% of the total CA1 field neurons 3 months after soman poisoning (Collombet et al., 2006).

Besides neuropathological sequels, behavioral changes have often been described after soman exposure. However, like neuropathology, the effects of soman on behavioral performances in animals have been mainly studied over a short period of time (2 weeks to 1 month) after intoxication. Thus, in rats, 2 weeks after soman poisoning, dose-dependent decrements were found in learning an operant alternation task (Modrow and Jaax, 1989), whereas deficits were reported in the differential reinforcement of low rate (DRL) acquisition (McDonough et al., 1989), in schedule-controlled behavior (Brezonoff et al., 1985; Hymowitz et al., 1985, 1990; Romano et al., 1985), maze learning (Raffaele et al., 1987), Morris water maze test (Brandeis et al., 1993; Filliat et al., 1999; Raveh et al., 2002, 2003), passive (Buccafusco et al., 1990) and active (Romano et al., 1985) avoidance behavior, novelty test and retention of brightness discrimination (Myhrer et al., 2005).

Many of the above cited tests especially address spatial mnemonic processes which are known to be supported notably by hippocampal formation (O'Keefe and Nadel, 1978). Interestingly, a correlation exists between the severity of neuropathological sequels in the hippocampus of soman-intoxicated animals and their behavioral scores (Filliat et al., 1999; Myhrer et al., 2005; Raveh et al., 2002, 2003). However, study of this correlation was limited to the first 3–4 weeks after poisoning. Since, as mentioned above, we recently demonstrated that a two-phase neurodegenerative process accompanied by delayed neuronal regeneration occurred in the hippocampus of soman-intoxicated animals, the purpose of this study was to investigate the long-term (up to 90 days) behavioral consequences of these degenerative and regenerative processes, notably on mnemonic cognitive processes.

It has been previously demonstrated that early body weight loss is an excellent non-lethal correlate of the severity of soman-induced convulsions (Churchill et al., 1985). Since neuropathology due to soman is related to seizure activity (see above) including seizures and motor convulsions, we demonstrated in a preliminary experiment that body weight loss is also a good index of the severity of neuronal degeneration in the hippocampus of intoxicated animals.

For possible correlation of mnemonic cognitive results with the severity of hippocampal neuropathology after soman administration, behavioral performances were analyzed taking into account the body weight loss measured in each intoxicated subject. The effects of soman intoxication on motor ability were studied by using the grip- and the rotarod-tests. Cognitive effects were assessed by two tests exploring spatial memory: the Morris water maze and T-maze tests. Those two tests are complementary: the Morris water maze test exploring long-term reference memory and the T-maze exploring short-term working memory. As described in the material and methods section, cognitive tests were performed using intoxicated animals selected from two independent experiments.

At last, it has been shown that after hippocampal insult, compensatory neuronal sprouting can lead to recovery of behavioral function within 2–5 months (Gage et al., 1983). To know whether such a phenomenon could participate, as well as neuronal regeneration, in the possible recovery of behavioral performance after soman poisoning, we also investigated neuronal sprouting in the hippocampus of animals 1 and 3 months after intoxication.

2. Materials and methods

2.1. Animals and soman exposure

Nine-week-old adult male B6D2F1/j@rj mice (Janvier Laboratories, France) were housed in cages under standard conditions of temperature (+24 °C) and humidity (50–60%), with a 12:12 h light/dark cycle and free access to water and standard laboratory chow. All the experiments were reviewed and approved by the Institutional Animal Care and Research Advisory Committee in accordance with French law and the main international guidelines.

Mice were subcutaneously injected with 1.2 LD_{50} of soman (110 μg/kg in 200 μl of saline solution) – provided by the ''Centre d'Etudes du Bouchet'' (France) – followed 1 min later by an intraperitoneal injection of atropine methyl nitrate (MNA) at 5.0 mg/kg body weight (200 μl in saline). MNA is a muscarinic receptor antagonist unable to cross the blood–brain barrier. Therefore, central nervous brain lesions are not affected by MNA treatment, while peripheral nervous system insult is partially prevented, leading to better animal survival. Control mice received intraperitoneal injections of MNA, but the soman injection was replaced by a subcutaneous injection of saline.

2.2. Preliminary experiment: correlation between body weight loss and hippocampal neuropathology after soman intoxication

Forty-nine animals were weighed (Mettler balance, accuracy: ±0.01 g), then intoxicated with soman and weighed again 3 days after neurotoxicant exposure. Just after this, mice were sacrificed for brain collection in order to quantify degenerating neurons in the hippocampal CA1 field as already described by Collombet et al. (2006). Briefly, pentobarbital anaesthetized mice were sacrificed by intracardiac perfusion of 0.1% (v/v) heparinated saline buffer and 4% formalin. The brains were collected and processed for paraffin embedding. Then, 6 μm coronal sections (−1.82 mm posterior to the bregma) were cut with a microtome and degenerating neurons were detected using hemalun–phloxin staining according to Lillie and Fullmer (1976). These neurons were numbered in the apex of field CA1 as previously described by Collombet et al. (2006). Then, for each animal, the number of degenerating CA1 neurons was plotted versus its weight loss determined on post-soman day 3, to examine a possible correlation between these two parameters.