

Soman-Induced Seizures and Related Brain Damage: How to Treat Seizures and Assess Their Effects Non-Invasively?

PIERRE CARPENTIER^{1*}, GUY TESTYLIER¹, VALÉRIE BAILLE¹, FRANK DHOTE¹, AGNÈS JOB², BENOIT POUYATOS², FABIEN PERNOT¹, ANNIE FOQUIN¹, CLAIRE DELACOUR¹, MURRAY HAMILTON³ AND FRÉDÉRIC DORANDEU¹

¹ Centre de Recherches du Service de Santé des Armées, Département de Toxicologie, La Tronche, France

² Centre de Recherches du Service de Santé des Armées, Département des Facteurs Humains, La Tronche, France

³ University of Denver, Denver, Colorado, USA

* Corresponding author:

Centre de Recherches du Service de Santé des Armées,

Département de Toxicologie,

24 Avenue des Maquis du Grésivaudan,

BP 87,

38702 La Tronche Cedex, France

Tel: (33) 476 63 69 85 / Fax: (33) 476 63 69 62 / E-mail: pierrecarpentier@crssa.net

ABSTRACT

Among organophosphorus nerve agents, soman is one of the most studied, being one of the most dangerous and the most difficult to counteract. When it does not kill rapidly, soman produces epileptic seizures and related brain damage which, if not treated immediately, may resist current therapies. The present review will address four points: (i) the medical issues raised by the central effects of soman poisoning; (ii) the mechanism of soman-induced brain damage; (iii) the use of antagonists of ionotropic glutamate receptors as anticonvulsants and neuroprotectants; and (iv) the search for new non-invasive methods to assess the progression of soman-induced brain damage.

INTRODUCTION

As proved by the Iran-Iraq war (1980-1988), the 1991 Gulf War and several terrorist attacks in Japan (1994, 1995), the potential for exposure to organophosphorus nerve agents, such as soman, sarin, tabun or VX, is still a matter of major concern. For nearly 30 years, our laboratory, among others, has focused on the study of the mechanisms involved in central nervous system poisoning and on treatment of seizures and related brain damage (SRBD) produced by soman. The first specific electroencephalographic (EEG) description of soman-induced seizures on monkeys was reported by Lipp [Lipp 1968, 1973]. Later, Petras provided clues that soman also produces damage in rat brain [Petras, 1981]. Soon after, our group published a more complete description of soman-induced SRBD [Lemerancier et al., 1983]. After these pioneering works, numerous studies worldwide repeated and confirmed the original findings in rodents and non-human primates.

The present review is written for readers who are not necessarily experts in the field and not necessarily fully aware of the literature concerning soman-induced seizures and SRBD. It provides a short presentation of the main issues in medical management of soman intoxication and a simplified account of the complex mechanisms involved in seizures and SRBD. In addition, it reports the contribution of our laboratory in the testing of new anticonvulsants and neuroprotectants - namely the non-competitive antagonists of the glutamatergic N-Methyl-D-Aspartate (NMDA) receptor - in view of enlarging the therapeutic time-window. Our recent effort towards finding new non-invasive markers of SRBD is also reported.

THE POTENTIAL THREAT OF SOMAN POISONING: AN INTRODUCTION

Similar to other nerve agents, soman acts as an irreversible inhibitor of acetylcholinesterase (AChE). The normal function of AChE is to hydrolyze acetylcholine (ACh) and thus regulate its action on specific receptors at the cholinergic synapses. Blockade of AChE by nerve agents results in a “hypercholinergic crisis” leading to a broad spectrum of toxic signs including hypersecretion, fasciculations, respiratory distress, cardio-vascular dysfunction, convulsive epileptic seizures and coma. Depending on the dose, death may occur within minutes after exposure, mainly due to respiratory failure. The medical issues linked to AChE inhibition by nerve agents can be worsened by a particular mechanism named “aging” that prevents reactivation of the inhibited enzyme. With soman, aging occurs within a few minutes thus contributing to the difficulty of efficient treatment.

The probability of survival can be increased by appropriate prophylaxis and therapies that are available in at least some countries. In case of an established threat, soldiers can be provided with pyridostigmine bromide at a dose that reversibly inhibits and, by occupying the catalytic site, protects 20-40% of the peripheral ChE from nerve agent-induced irreversible inhibition [NATO, 2006]. In the event of poisoning, a standard emergency treatment, often packaged in auto-injectors (single or multiple), is available, composed of an anticholinergic drug (e.g., atropine sulphate) and an AChE reactivator (an oxime). In some countries, an anticonvulsant benzodiazepine (currently mostly diazepam or its prodrug avizafone) is also issued. It can either be incorporated into the same auto-injector, as in France, or issued in a separate autoinjector. The military doctrine prescribes intramuscular (i.m.) self-injection of one auto-injector at the first signs of poisoning. If no improvement is observed, a second one should be administered 10—15 min later. In some armed forces, a third auto-injector may be available and administered. The doctrine for follow-up injections varies among nations, but both the second, and possibly third, injection most probably will not be self-injected by the poisoned individual. Rather these will be given by either a buddy-aid or some kind of health care provider. With such treatments, victims may survive even supralethal doses of soman. For instance, *Cynomolgus* monkeys, pretreated with pyridostigmine and subsequently treated with two French auto-injectors (atropine sulphate/pralidoxime methane sulfonate/diazepam), survived intoxication by 8 x LD₅₀ of soman [Lallement et al., 1999].

However, life-saving medical intervention after soman poisoning is not sufficient, as survivors may experience continuing seizures and thus be prone to develop SRBD. Indeed, after a convulsive dose of soman, spiking activity appears in the EEG tracings in less than 10 min, rapidly progressing into status epilepticus (SE) that lasts for hours [Carpentier et al., 1990; McDonough and Shih, 1993; Carpentier et al., 1994]. Soman-induced SRBD can be detected in numerous sensitive brain regions (e.g., hippocampus, amygdala, piriform cortex). In the first few hours after poisoning, extensive cerebral edema and neuronal changes appear. This leads, 24 to 72 h post-exposure, to significant cell loss that may account for profound long-term neurological deficits [Philippens et al., 1992; Filliat et al., 1999; Myhrer et al., 2005]. Indeed, owing to the fact that pyridostigmine and oximes poorly penetrate the blood-brain barrier, they do not provide any significant protection against seizures and SRBD. Although atropine sulphate and diazepam could lead to substantial central protection, it is not always complete [McDonough and Shih, 1997], especially with highly toxic doses of the nerve agent. For instance, monkeys poisoned with 8 x LD₅₀ of soman displayed seizures and SRBD despite prophylaxis and the use of the emergency drug treatment [Lallement et al., 1999]. Comparable results were reported in soman poisoned rats [Philippens et al., 1992]. Protection failure may also occur when the full set of treatments (i.e., the entire contents of 1 to 3 auto-injectors) is either incomplete or too late, a likely situation in the field. Above all, multiple observations indicate that, after 20—40 min, soman-induced SE becomes refractory to most of the conventional antiepileptic drugs [McDonough and Shih, 1997]. Interestingly, this property is not only shared by nerve agent-induced seizures but also by self-sustaining SE in many other experimental models and clinical situations [Chen and Wasterlain., 2006; Mazarati et al., 2006].

Therefore, it appears that (i) the use of protective drugs must be initiated as soon as possible after soman exposure, and (ii), there is a clear need to explore the capacity of new drugs capable of blocking seizures and at least minimizing SRBD when they are administered more than 1 h after the intoxication. Delays in the initiation of treatment beyond the present therapeutic window of opportunity can be expected due to confusion in battle or in the aftermath of a terrorist incident.

MECHANISMS OF SOMAN-INDUCED SRBD: AN OVERVIEW

It is generally thought that soman-induced SRBD is mainly due to the unregulated activity of the excitatory neurotransmitter glutamate (Glu). While ACh is not cytotoxic by itself [Sloviter and Dempster, 1985], Glu-induced excitotoxicity is well recognized [Olney et al., 1986]. Under repetitive depolarization, Glu is released in excess and overstimulates its various receptors. These can either be ionotropic, such as the NMDA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or kainate receptors, or metabotropic receptors, of which eight subtypes are known. To date, although the molecular basis of Glu toxicity remains uncertain, there is a general agreement that NMDA receptors play a key role in mediating at least some aspects of Glu neurotoxicity [Kew and Kemp, 2005; Gardoni and Di Luca, 2006]. The resulting intracellular calcium overload is particularly important as it induces supplementary depolarization and overactivation of enzymes (lipases, proteases, endonucleases, kinases, phosphatases) that can directly damage cell membranes, cytoskeleton or organelle structure and functions (e.g., mitochondria: [Ankarcrona et al., 1995; Cock et al., 2002]), or promote oxidative stress [Pazdernik et al., 2001] or inflammation [Svensson et al., 2001; Williams et al., 2003; Dhote et al., 2007]. All these events can eventually lead to cell death.

The respective involvement of ACh and Glu can be summarized in the schematic time-frame describing the successive events leading to soman-induced SRBD [McDonough and Shih, 1997; Shih and McDonough, 1997], which is in fact a continuum with no absolute boundaries (figure 1).

The early cholinergic phase: the first approximately 20 min of soman-induced seizures.

In response to soman-induced inhibition of AChE, an almost immediate increase in brain ACh is recorded [Shih, 1982; Fosbraey et al., 1990; Lallement et al., 1992; Tonduli et al., 1999]. Cholinergic hyperstimulation, probably with a contribution of Glu on its AMPA receptors [Lallement et al., 1994], immediately initiates seizures that rapidly lead to an “impending” SE [Chen and Wasterlain, 2006]. Changes in the tissue or intracellular levels of other neurotransmitters (norepinephrine, dopamine, serotonin, GABA) have also been reported [Fosbraey et al., 1990; McDonough and Shih, 1997]. During this early phase, seizures can be efficiently terminated and SRBD avoided by drugs that, alone or in combination, can:

- (i) block the muscarinic receptors [e.g., Shih et al., 1991; McDonough et al., 1995; McDonough et al., 2000; Carpentier et al., 2000; Shih et al., 2003]
- (ii) diminish the release of ACh:
 - a. Agonists of central adrenergic α -2- receptors that are effective as pretreatments and as treatment only in combination with atropine sulphate [Loke et al., 2002].
 - b. Adenosine A1 receptor agonists that proved effective against some nerve agents but have only been tested as a very early (1 min) treatment [e.g., van Helden and Bueters, 1999].
 - c. D1 dopamine receptor antagonist when delivered prior to poisoning [Bourne et al., 2001]; however, in this case, other mechanisms might be involved owing to the known interactions between D1 receptors and Glu ionotropic receptor trafficking [Fiorentini et al., 2003; Mangiavacchi and Wolf, 2004];
- (iii) enhance the inhibitory GABA_A neurotransmission [e.g., Clement and Broxup, 1993; Lallement et al., 1997; Lallement et al., 2000; Myhrer et al., 2006a, b] ; or
- (iv) block the AMPA receptor [e.g., Lallement et al., 1994].

The transitional phase: approximately 20-30 min after seizure onset.

During this phase, recruitment of the excitatory Glu system progressively supersedes the effects of cholinergic overstimulation [Wade et al., 1987; Lallement et al., 1991a; 1991b; 1991c; 1992], which explains the progressive loss of the efficacy of “pure” antimuscarinics [McDonough and Shih, 1997]. The efficacy of benzodiazepine also tends to diminish, a feature shared with SE of different origins in which the internalization of GABA_A receptors was suggested as a possible cause [Chen and Wasterlain, 2006]. This may thus be involved in soman-induced SE as well. Primarily through overstimulation of the NMDA receptors, soman-induced seizures gradually change from an “impending” to an “established” type of SE and SRBD begins to develop.

The Glu phase: approximately 30-60 min after seizure onset.

At approximately 30 minutes in experimental animals, SE becomes self-sustaining, SRBD is distinct and pharmaco-resistance develops. This is well supported by experimental and clinical studies [Chen and Wasterlain, 2006]. Although ACh levels generally peak 50 min post-soman exposure before declining [Shih, 1982; Fosbraey et al., 1990; Lallement et al., 1992; Tonduli et al., 1999], their importance appears to be reduced because at this point, the Glu excitatory system is the main contributor to the maintenance of seizures and development of SRBD. Interestingly, when SE becomes self-sustaining, NMDA antagonists are almost the only drugs that are effective in stopping seizures and development of SRBD, not only in soman poisoning (see next section), but also in many other cases of self-sustaining SE of various origins. Consequently, the mechanisms of self-sustaining SE often appear to be unrelated to the trigger event [Mazarati et al., 2006]. The fact that, in soman poisoning and other pathologies, common mechanisms (Glu involvement) could lead to self-sustaining SE suggests that medical countermeasures against soman-induced seizures could benefit from the work conducted in other areas of epilepsy research.

The subacute period: beyond 60 min.

This late period after 60 minutes is marked by

- (i) a progressive decline in seizure intensity;
- (ii) an expansion and maturation of structural changes culminating in widespread cell damage;
- (iii) a decrease in Glu release [Wade et al., 1987; Lallement et al., 1991a; Lallement et al., 1991b; Lallement et al., 1991c; Lallement et al., 1992] and, most importantly,
- (iv) a decrease in the anticonvulsant and neuroprotective efficacy of the NMDA antagonists (see next section).

It is important to stress that knowledge of the neurochemical changes is limited to the very first hour of poisoning [Wade et al., 1987; Lallement et al., 1991a].

The failure of anti-NMDA compounds to either block seizures and/or prevent or repair cell damage after 60-90 min might stem from several causes that remain theoretical in the case of soman poisoning. These causes include:

- (i) the penetration of non-competitive antagonists into the brain and their action in the receptor channel may be reduced due to changes in circulation and acid/base balance,;
- (ii) Glu receptors might no longer be within reach of the NMDA antagonists due to changes in membrane fluidity [Stalc et al., 1989; Grange-Messent and Bouchaud, 1994] and/or to changes in the mobility, trafficking or distribution of the receptors [Wang et al., 2004; Besshoh et al., 2005; Chen and Wasterlain, 2006; Groc et al., 2006];
- (iii) changes in the sub-unit composition of the NMDA receptors might be involved although differences in sensitivity to antagonists are very small [Paoletti et al., 2007];
- (iv) other mechanisms might be involved, including other neurotransmitter systems or “non-synaptic” mechanisms [Jefferys and Haas, 1982; Bikson et al., 2002], as well as neuro-inflammation that could affect Glu transmission [Leonoudakis et al., 2004]; and finally,
- (v) the course of cell degeneration, whatever the mechanism, may become irreversible.

Other possible contributing factors to soman-induced SRBD

During the first hours of soman seizures, most of the metabolic changes recorded, i.e., increase in glucose use, partial pressure of tissue oxygen and lactic acidosis, hyperglycemia, as well as cardiovascular changes (increase in cerebral blood flow, hypertension), indicate that the brain is globally hypermetabolic and able to adjust the energy supply to the demand [McDonough et al., 1983; Brezenoff et al., 1984; Pazdernik et al., 1985; Lynch and Glenn, 1987;

Bataillard et al., 1990; Shih and Screamin, 1992; Goldman et al., 1993; Letienne et al., 1999]. By fuelling neuronal firing, these conditions might contribute to accelerating the excitotoxic cell degradation. In human SE, a similar phase is described as “phase I” or the “compensatory phase” [Ichai et al., 1996]. In the majority of brain structures, both cerebral blood flow and metabolism similarly increased within minutes after seizure onset. Conversely, in a limited number of areas, soman exposure induced an increase in metabolism that was not matched by the necessary increase in regional blood flow, thus inducing a “relative” ischemia that may account for some local cellular damage.

The entry of hazardous substances through a leaky blood-brain barrier has also been implicated during the most acute period [Carpentier et al., 1990].

Later, at least some of the events described in the “phase II” (or “decompensatory” phase) of SE in humans may be progressively involved in soman poisoning [Ichai et al., 1996]. These events include (i) loss of auto-regulation of the cerebral blood flow with deleterious consequences on the cerebral perfusion pressure; (ii) increase in the intracranial pressure through different mechanisms including cerebral edema; and (iii) establishment of hypometabolic, hypoglycemic and hypoxic/ischemic/anoxic conditions. These situations, and possibly the soman-induced neuroinflammatory reaction, may be actual causes of cell death or may be synergistic with other pathways [Zimmer et al., 1997; Svensson et al., 2001; Williams et al., 2003; Baille et al., 2005; Svensson et al., 2005; Dhote et al., 2007]. A recent ultrastructure study of the neuropathology of soman intoxication showed an amazingly large panel of structurally different forms of cell death in the brain of soman-intoxicated mice [Baille et al., 2005]. While pure apoptosis appeared extremely rarely, and it certainly was not a major feature in this murine model of SE, pictures of autophagy and a number of hybrid forms ranging between necrosis and apoptosis were detected. It would be surprising if such diversity resulted from a single process.

NON-COMPETITIVE NMDA ANTAGONISTS IN SOMAN POISONING: A SHORT HISTORY

As mentioned above, current anticonvulsant/neuroprotectant treatments do not work very well 20—40 min after soman poisoning. Since after about 20 min NMDA receptors are involved in the mechanisms of seizures and SRBD, it was thought that antagonizing NMDA receptors could be suitable to expand the anticonvulsant/neuroprotectant time-window in soman poisoning. We will first review the pioneering work conducted with MK-801 and then focus on other non-competitive NMDA antagonists, i.e., TCP, GK-11 and ketamine, which have been tested in our laboratory during the last 15 years. Other compounds interacting with NMDA receptors (e.g., memantine, dextromethorphan, felbamate, and HU-211) or having dual potential as ACh and Glu antagonists have also been tested [Dematteis et al., 1997; Nyberg et al., 1997; Raveh et al., 2003; Filbert et al., 2005].

MK-801 (dizocilpine): the pioneering work

MK-801, a selective non-competitive antagonist of the NMDA receptor, was used successfully as a potent anticonvulsant and neuroprotectant in various seizure models [Ormandy et al., 1989; Clifford et al., 1990; Johnson and Jones, 1990; Walton and Treiman, 1991]. In guinea-pigs intoxicated by a convulsing dose of soman (and in the presence of pyridostigmine, oxime and atropine sulphate), MK-801, administered 5, 50 or 120 min after seizure onset, was shown to terminate seizure activity, to increase the survival rate, and to prevent SRBD [Braitman et al., 1989; Sparenborg et al., 1991; Sparenborg et al., 1992].

However, MK-801, in the absence of atropine sulphate, was found to potentiate the lethal respiratory effects produced by soman [Shih, 1990; Shih et al., 1991; McDonough and Shih, 1993]. Dizocilpine has been used as a recreational drug and induce motor syndrome, psychomimetic effects, impair learning and memory, act as a proconvulsant and has neuropathological sequelae [Burns and Lerner, 1976; Kemp et al., 1987; Tricklebank et al., 1989; Filliat and Blanchet, 1995; see also Carpentier et al., 1994; 2001b]. All these undesirable side effects render MK-801 unsuitable for use in treating human intoxication.

TCP (thienyl phencyclidine): experiments in rodents

TCP, like MK-801, shows high affinity and selectivity for the NMDA receptor [Kemp et al., 1987; Maragos et al., 1988]. In guinea-pigs intoxicated by soman in the presence of pyridostigmine and atropine sulphate, TCP,

administered 5 to 60 min post-challenge, was shown to completely stop ongoing seizures within minutes after injection [Blanchet et al., 1992; Carpentier et al., 1994; 2001b; de Groot et al., 2001]. This seizure cessation was accompanied by a 100% survival rate, good clinical recovery and excellent neuroprotection. TCP also provided impressive beneficial effects in shuttle box and Morris water maze behavioral tests [de Groot et al., 2001]. When administered later, 90 or 120 min after seizure onset, the beneficial effects of TCP were clearly reduced.

In the presence of atropine sulphate, TCP, similar to MK-801, acts as an anticonvulsant, is neuroprotective and eliminates lethal effects in soman poisoning. However, in contrast with MK-801, TCP failed to potentiate soman-induced respiratory distress, even in the absence of atropine sulphate [Carpentier et al., 1994]. Moreover, TCP elicits much less locomotor impairment, abnormal behavior, proconvulsant activity and neuropathology [see Carpentier et al., 2001b]. Despite these positive experimental results, TCP has never been tested in non-human primates and has never been considered for clinical use, owing to its structural similarity to the phencyclidine, also known as PCP and a commonly abused drug.

GK-11 (gacyclidine): French experiments in non-human primates

GK-11 was first tested by our group as an adjuvant therapy to the existing emergency treatments in non-human primates (*Cynomolgus* monkeys) [review in Lallement et al., 1999]. In a first experiment, monkeys were pretreated with pyridostigmine, heavily intoxicated with 8 x LD₅₀ of soman and then immediately treated with the man-equivalent content of two French auto-injectors (atropine sulphate, oxime, diazepam). This protocol proved effective against the lethal effects of soman, but did not entirely restore normal EEG activity nor totally prevent SRBD. Additional administration of GK-11, 10 min post-challenge, induced a transient ataxia but controlled the convulsions, improved respiration and totally eliminated SRBD. In another experiment, the animals were pre-treated and intoxicated as above, but received only one man-equivalent auto-injector and GK-11 as late as 45 min post-challenge. In spite of these unfavourable conditions, GK-11 still proved an excellent antilethal, anticonvulsant and neuroprotectant compound. In the absence of pretreatment and after tri-therapy (atropine sulphate/oxime/diazepam) given 30 min post-soman exposure, GK-11, when given an additional injection 10 min later, again proved fully effective. However, when the tri-therapy was delayed to 45 min post-challenge, the addition of GK-11 10 min later was unable to counteract the effects of soman. Other results from soman-intoxicated rats indicate that GK-11 may prevent severe neuronal changes in some (but not all) brain areas, even when administered 3 h after seizure onset [Bhagat et al., 2005].

All in all, GK-11 appeared promising to supplement the current emergency polymedication. Unfortunately, Ipsen-Beaufour Laboratories, current owner of the molecule, decided to stop the clinical development of the compound after GK-11 failed to significantly improve the outcome of brain and spinal cord injuries (the main clinical indications for which GK-11 was developed).

Ketamine: French experiments in guinea-pigs

Ketamine, mostly available as a racemic mixture or, in some countries, as the S (+) isomer, is at present the only commercially available injectable NMDA antagonist licensed for human use. It is commonly used as an anesthetic in emergency care and considered in some countries as a third-line treatment of refractory SE in humans [for references see Dorandeu et al., 2003; Chen and Wasterlain, 2006]. Ketamine-induced respiratory depression is rare, at regular dosage, in humans. Ketamine-induced psychomimetic side-effects (of little relevance when dealing with a life-threatening situation like refractory SE) can be clinically prevented or reduced by either a combined administration with benzodiazepines (for instance) or the use of S-ketamine. S-ketamine can be used at lower doses, as its affinity for the NMDA receptor is four times greater than that of the R (-) enantiomer.

A previous attempt to evaluate ketamine during soman-induced seizures failed to find any beneficial effects of a single low dose injected very early after challenge [Shih et al., 1999]. Since NMDA receptor antagonists usually prove more efficient when administered later in the course of seizures, new experiments were then undertaken [Dorandeu et al., 2005, 2007] in which ketamine, or S-ketamine, were repeatedly administered every 30 min in association with atropine sulphate to guinea-pigs poisoned by 2 x LD₅₀ of soman. Animals were intoxicated in the presence of pyridostigmine and atropine methyl nitrate (a peripherally-acting antimuscarinic) to ensure sufficient survival for observation of long lasting seizures. Repeated injections of ketamine appeared necessary because of the short action of the drug. In these conditions, a sub-anesthetic dose of ketamine (10 mg/kg) exerted its best protective

activity against soman-induced seizures, SRBD and lethality when it was administered 6 times, the first injection being performed 30 min post-soman. The efficacy was greatly reduced when the first injection was delayed to 60 min. In contrast, increasing the ketamine dose to anesthetic levels (40-60 mg/kg in the guinea pig) was successful at this delayed time point [Dorandeu et al., 2005]. S-ketamine administration also showed similar protective effects with a dose (15 mg/kg) two or threefold lower than those used with the racemic ketamine [Dorandeu et al., 2007].

Therefore, combined with atropine sulphate, ketamine or S-ketamine proved effective by extending the therapeutic time-window to at least 60 min. This gain in time might be crucial to save lives and minimize soman-induced SRBD. Further studies will aim at better defining treatment schemes and/or associations for successful and safe management of more prolonged SE. Experiments in higher species (swine; non-human primates) are also required.

NON-INVASIVE MARKERS OF SRBD: A CRUCIAL NEED

In the laboratory, the assessment of brain damage through histopathology is time-consuming, requires animal sacrifice and provides only “snapshots” of structural changes at a given time. Non-invasive methods are needed for monitoring the evolution of SRBD over time (where each animal can be its own control) and, ultimately, for clinical use. In soman poisoning, indirect methods for the diagnosis of brain damage exist. They rely on previously established relationships between the histological status of the brain and clinical observations (occurrence of convulsions), analysis of EEG tracings (development of electrographic seizures) or measurement of blood ChE activity (degree of inhibition). The potential of three new non-invasive tools to assess SRBD in soman poisoning are presented below.

EEG delta activity

EEG tracings can give indications on the incidence, distribution and severity of the cerebral insult [Carpentier et al., 2000; 2001b]. However, duration and intensity of EEG seizures could differ according to the pharmacological environment or the animal species [Carpentier et al., 2001b]. Notably, some drugs proved effective neuroprotectants without altering the course of clinical and EEG seizures [Filbert et al., 2005].

Digitized EEG recordings allow more detailed and quantitative analysis of the EEG power in the broad spectrum (total power) or in specific frequency bands. In soman poisoning, following initial observations [e.g., Philippens et al., 1992; Melchers et al., 1994], a series of independent experiments in rodents and monkeys established the EEG spectrum correlates to SRBD [Lallement et al., 1998; McDonough et al., 1998; Carpentier et al., 2000; 2001a; 2001b; de Groot et al., 2001]. Despite experimental disparity (animal models, intoxication protocols, pharmacological environment, period/duration of analysis, etc.), the results all showed a significant increase ($p < 0.01$ or 0.05) in the relative contribution of the delta band (the lowest frequency range: 0.1 to 5.0 Hz) to EEG total power. Within the first hour, this increase was associated with the peak of seizure activity. In the following hours until 48 h post-challenge, delta relative power still remained abnormally high although seizure activity declined and eventually disappeared. Within this period, soman-induced SRBD is known to start, progress and mature. It was then proposed that an increase of the relative delta power might be a real-time marker for the concurrent development of SRBD. This hypothesis was strengthened by the facts that (i) animals that did not experience seizures never displayed delta changes or SRBD; (ii) timely treatment with NMDA antagonists arrested EEG seizures, normalized delta activity and prevented SRBD; and (iii) an increase in delta activity at 24 h positively correlated with the importance of SRBD. Beyond 48 h, the delta/SRBD relationship is less clear with data differing between the various experiments. For instance, in poisoned rats, Philippens et al. (1992) found abnormally high delta activity for 6-7 days, while McDonough et al., (1998) showed that it eventually normalized, starting on the third day.

The use of EEG delta activity to predict SRBD has some drawbacks: it cannot locate the damaged cerebral areas or determine how many areas are affected and it may be modified by various treatments [Lallement et al., 1998]. Nonetheless, and because of their apparent universality from rodents to non-human primates, the experimental findings lend considerable support to the possibility that analysis of EEG power spectra may have important diagnostic applications in humans.

Diffusion-weighted magnetic resonance imaging

Since soman-induced SRBD is always accompanied by concomitant development of cerebral edema, it can be speculated that edema detection may be a marker for SRBD. In this respect, diffusion weighted-magnetic resonance imaging (DW-MRI), a unique form of MRI contrast based on the translational diffusion motion of water molecules, has emerged as a sensitive, non-invasive method to detect the structural changes accompanying cerebral edema [references in Testylier et al., 2007]. Maps representing the “apparent diffusion coefficient” (ADC) of water can be derived from DW-MRI measurements. A decrease in ADC is classically assigned to “cytotoxic” cerebral edema resulting from abnormal uptake of extracellular water by brain cells. Conversely, an increase of ADC is classically assigned to extracellular cerebral edema mainly of “vasogenic” origin.

In our experiments performed in mice poisoned with a convulsive dose of soman, a decrease of post-soman ADC was already prominent at 3 h and most pronounced the day after. This indicates that soman-induced cerebral edema is predominantly cytotoxic during this period [Testylier et al., 2007]. More importantly, a correlation was demonstrated between local ADC changes and the severity of lesions. For instance, the amygdala showed the greatest ADC drop (significant at $p < 0.05$ at 3 h and $p < 0.01$ at 24 h) and the most severe damage, while the hippocampus displayed the weakest ADC drop (non significant) and the least severe lesions. Moreover, when the intoxicated animals were immediately treated with atropine sulphate and diazepam, they did not convulse and showed neither brain lesions nor drops in ADC 24 h after poisoning.

In spite of some discrepancies in the results (likely related to differences between the experimental designs), we and our Canadian colleagues [Bhagat et al., 2001; 2005] reached the same conclusions: DW-MRI appears very promising as a sensitive non-invasive method for following the progression of SRBD in soman poisoning, to differentiate brain regions based on the severity of lesions and to assess the efficacies of neuroprotectants.

Distortion product otoacoustic emissions

Since the same neurotransmitters, ACh and Glu, play crucial roles not only in soman-induced seizures and SRBD but also in auditory function [see Job et al., 2007], it was postulated that (i), soman should have effects on the auditory system, and (ii), measuring hearing changes may serve as a biomarker of soman-induced central effects. In order to assess these hypotheses, we used an audiometric technique that relies on the contractile properties of the cochlear outer hair cells, allowing the measurement of distortion product otoacoustic emissions (DPOAEs). Retrograde wave sounds are generated in response to tonal sound stimuli. A microphone placed in the external auditory canal can record their intensity. When two primary pure tones (f_1 and f_2) are presented simultaneously with a ratio f_2/f_1 of between 1.2 and 1.3, distortion products are emitted, the largest occurring at the frequency $2f_1 - f_2$. Since the amplitude of DPOAEs depends on the integrity of outer hair cells, they have been used to evaluate cochlear vulnerability in clinical settings [Job and Nottet, 2002; Job et al., 2004] and the ototoxicity of various chemicals [Lataye et al., 2003; Pouyatos et al., 2005].

In our experiments in rats [Job et al., 2007], DPOAEs (frequency range: 2-4 kHz; stimulus intensity: 70/70 dB) were measured under anesthesia 8 d before (baseline) and either 4 h or 24 h after exposure to a moderate dose of soman (45 $\mu\text{g}/\text{kg}$) producing various symptoms, from almost no signs (grade 1) to long-lasting convulsions (grade 4). Immediately following DPOAE measurement, the animals were sacrificed for either brain histology or measurement of ChE activity in whole blood or brain. At 4 h post-challenge, the intoxicated rats showed a decrease of DPOAEs correlating with the severity of intoxication: the greatest drop in DPOAEs (about – 5dB below the control value; difference significant at $p < 0.01$) was recorded in grade 4 rats displaying the most severe symptoms, the highest inhibition of cerebral ChE and extensive brain damage. In less affected animals (with smaller ChE, no long-lasting convulsions, no detectable brain damage) DPOAEs were not significantly modified. At 24 h, DPOAEs normalized in all the rats.

These preliminary findings suggest that, at least during the first hours, DPOAEs might be useful to confirm clinical observations and blood AChE dosage after systemic poisoning, to quickly and non-invasively evaluate the severity of the intoxication, and to assess the potential for late neurological damage. Even if the method may not appear to provide specific information about SRBD, it may answer the question of whether damage is in progress or not. However, due to DPOEA normalization at 24 h, the method does not seem to be of interest for monitoring further progression of the damage or for evaluation of the neuroprotectant effects of a medication.

Interestingly, a significant relationship ($p < 0.05$) was also found between the pre-soman baseline and the severity of symptoms produced after the intoxication: baseline DPOAEs were the lowest in the rats that, after the intoxication, displayed the highest brain ChE inhibition, convulsions and SRBD (Grade 4). These amazing findings suggest that DPOAEs might be used as a predictor of susceptibility to convulsions and SRBD. If such observations could be reproduced in clinical situations, DPOAEs might be used to detect the individuals most sensitive to soman-induced central effects.

Conclusion on the non-invasive markers of SRBD

All in all, experimental data suggest that EEG spectrum, DW-MRI and DPOAEs might provide interesting non-invasive tools for evaluating the acute impact of soman intoxication on the brain and the effect of a medication. In the experimental and clinical fields, such methods, if proved reliable, might supplement the existing ones in order to increase the accuracy and potency of the prognosis/diagnosis of soman intoxication and of treatments. The DPOAE method needs no particular preparation of the subject, and thus can be used in pre-hospital settings. Theoretically, the low weight and bulk of the device allows measurements to be performed anywhere it is needed. In the military context, these characteristics and the fact that DPOAEs seem a good marker of central events during the most acute phase of an intoxication, make the method extremely interesting for early diagnostic of SRBD, thus allowing rapid intervention. In contrast, the use of EEG and DW-MRI in humans will be more difficult in the field as they require essentially non-mobile equipment and the highly specialized human and technological environment of a hospital. Nevertheless, later in the course of the poisoning, EEG (for long periods) and DW-MRI (at selected time points) might serve to assess the efficacy of neuroprotectants.

GENERAL CONCLUSIONS

Substantial data have been accumulating over the last decades about soman-induced seizures and SRBD. However, much remains to be done. For instance, the intracellular cascades of events leading to cell death remain almost entirely unknown. Such knowledge may provide targets for new neuroprotective strategies. Similarly, although ketamine seemingly constitutes a significant progress, much effort is needed to enlarge the therapeutic window of opportunity and reduce the potential side-effects of long-term treatments. Finally, although theoretically promising, the aforementioned diagnostic methods for the non-invasive assessment of soman-induced SRBD require further testing to evaluate their limits, robustness and above all their potential clinical application.

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FIGURES

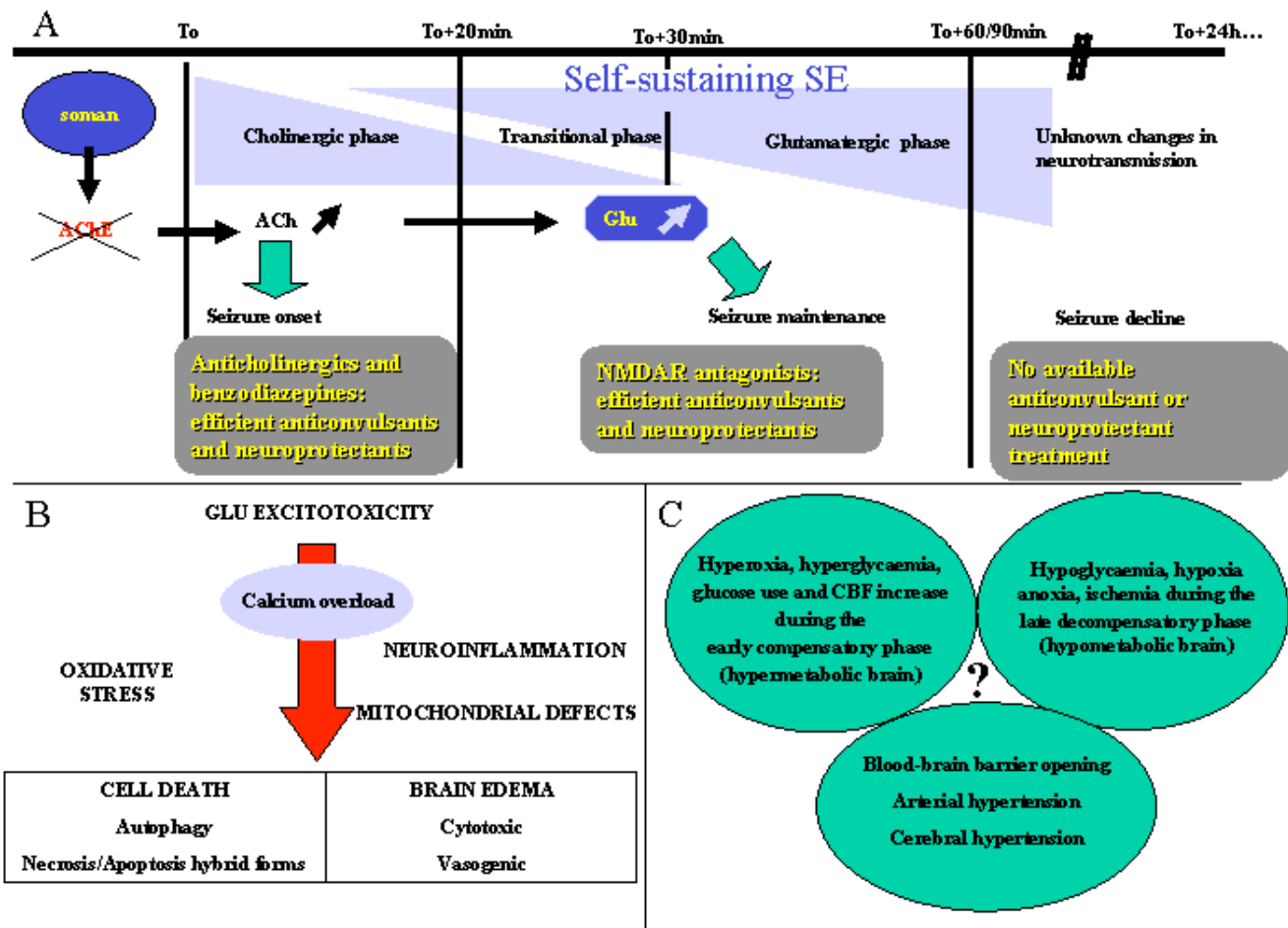


Fig.1 : Soman-induced SRBD.

A: Acute neurochemical/electrographical events and treatments.

The diagram shows how inhibition of AChE by soman immediately produces a hypercholinergic situation responsible for seizures. This early phase can be counteracted by atropine and benzodiazepines. A second phase occurs as Glu is progressively recruited, maintaining the seizures. During this phase, NMDAR antagonists have proved efficient anticonvulsants and neuroprotectants. Beyond 60/90 min, seizure activity progressively declines while SRBD continues. During this phase, the mechanisms remain essentially unknown and no neuroprotectant medication is available so far.

B: Glu-mediated excitotoxicity and related events.

The diagram shows that the essential factor of SRBD is Glu which, when uncontrolled, can produce calcium overload and a subsequent series of cellular or tissular events leading to cell death and edema.

C: Other possible physiopathological contributors

Soman produces a number of physiopathological effects. Potential contributors to SRBD are listed.

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