Soman-Induced Neuro-Inflammatory Reaction in Mouse Brain. Some Effects of a Combination of Atropine and Ketamine

Dr. Franck Dhote, Dr. André Peinnequin,
Dr. Pierre Carpentier, Dr. Valérie Baille,
Ms. Claire Delacour, Ms. Annie Foquin,
Pr. Guy Lallement, Dr. Frédéric Dorandeu

Centre de Recherches du Service de Santé des Armées « Emile Pardé » -CRSSA-
24, avenue des maquis du Grésivaudan
38702 La Tronche Cedex
France

Email : franckdhote@crssa.net / andrepeinnequin@crssa.net / pierrecarpentier@crssa.net / valeriebaille@crssa.net / cdelacour@crssa.net / afoquin@crssa.net / fdorandeu@crssa.net

ABSTRACT

Organophosphorus nerve agent (OP) poisoning induces death through different mechanisms including respiratory and cardiovascular major dysfunctions. Epileptic seizures, status epilepticus and seizure-related brain damage (SRBD) complicate the clinical picture. Recently different publications showed that neuro-inflammatory events take place during OP-induced seizures but their role in SRBD and post-status epilepticus epileptogenesis is not clear. Antagonists of the NMDA ionotropic glutamate receptors are currently the only drugs able to arrest seizures and provide neuroprotection when treatment is initiated up to one hour after poisoning. We recently reported that racemic ketamine, or the more active isomer S(+) ketamine, in combination with atropine sulphate (AS), could counteract lethality, seizures and SRBD in soman poisoned guinea-pigs. Moreover peripheral anti-inflammatory properties of ketamine have been described. To complement former studies restricted to shorter time courses, we undertook a quantitative RT-PCR analysis of the brain genic response to soman (IL-1β, TNFα, IL-6, ICAM-1 and SOCS3) up to 7 days after poisoning in a mouse model of severe convulsions and neuropathy (HI-6, 50 mg/kg i.p. 5 min prior to 172 µg/kg soman, s.c.). Changes in mRNA levels were quantified in hippocampus and cortex. We also assessed 48h post intoxication the effects of two regimens of racemic ketamine combined with AS (10 mg/kg, i.p.) on the mRNA levels of the same genes, on the related protein levels as well as on the protein levels for RANTES and KC, two chemokines, VCAM1 and IL-10. Six injections of a sub-anaesthetic dose (25 mg/kg, i.p.) were performed every half-an-hour starting 30 minutes post-poisoning. When treatment initiation was delayed to 1 h post-challenge, ketamine (100 mg/kg) was injected at 60, 120 and 180 minutes. In response to soman intoxication an important and highly significant increase of the five mRNA levels was recorded in cortex and hippocampus. In the cortex, the activation was generally detected as early as 1 h post-intoxication with a peak response recorded between 6 and 24 h. In the hippocampus, the mRNA increase was delayed to 6 h post-soman and the peak response observed between 24 h and 48 h. After peaking, the response declined (except for ICAM-1 in the hippocampus) but remained elevated, some of them significantly, at day 7. With both treatment regimens, the ketamine-AS combination was able to counteract the changes in mRNA and related proteins provoked by poisoning. In conclusion, the present study indicates a quick neuro-inflammatory gene response that does not subside over 7 days. The effects of ketamine combined with AS on the neuro-inflammation remain to be clearly understood.

Dhote, F.; Peinnequin, A.; Carpentier, P.; Baille, V.; Delacour, C.; Foquin, A.; Lallement, G.; Dorandeu, F. (2007) Soman-Induced Neuro-Inflammatory Reaction in Mouse Brain. Some Effects of a Combination of Atropine and Ketamine. In *Defence against the Effects of Chemical Hazards: Toxicology, Diagnosis and Medical Countermeasures* (pp. 2-1 – 2-20). Meeting Proceedings RTO-MP-HFM-149, Paper 2. Neuilly-sur-Seine, France: RTO. Available from: http://www.rto.nato.int.

1.0 INTRODUCTION

In the chemical warfare arsenal, the organophosphorus (OP) nerve agents (NA) are the most dangerous agents known. They may be feared during combat situations (e.g. Iran-Irak war (1980-1988) and the second Gulf war in 1991), as well as terrorist attacks (Japan, 1994-1995 [1]). They act as potent irreversible inhibitors of cholinesterases (ChEs) in both central (CNS) and peripheral nervous systems and induce an immediate hypercholinergic crisis responsible for most of the pathological responses to the poisoning. Among NA, soman is considered as a major threat, and particularly studied because of the difficulty of the treatment. As with other NA, depending upon the dose, exposure to soman can produce hypersalivation, respiratory distress, cardiovascular dysfunction, coma and rapid death. In the brain, the excess of acetylcholine triggers seizure activity and leads to the secondary recruitment of the excitatory glutamate (Glu) system. Through overstimulation of Glu receptors, including the ionotropic N-methyl-$_D$-aspartate (NMDA) receptors, Glu is thought to play a prominent role in the long-lasting maintenance of seizure activity (Status epilepticus, SE) and in the build-up of irreversible seizure-related brain damage (SRBD).

When injured the brain is usually the site of an inflammatory reaction that was particularly well studied in acute affections such as cranial trauma, cerebral ischaemia or epilepsy [2, 3]. Inflammation has usually an important repairing function, but frequently in the CNS it rather appears to be the cause of damage and does not fulfil this repairing function [4]. Glial cells (microglia and astrocytes) are key mediators of the immune response in the CNS. Microglia is closely related to macrophages and undergoes dramatic morphological and functional changes after CNS trauma or excitotoxic lesion and can be directly stimulated by excitatory neurotransmitters such as Glu, inducing the production of inflammatory mediators such as cytokines [5]. Amongst inflammatory mediators, pro-inflammatory cytokines (TNFα, IL-1β, IL-6...) play an important role by supporting the activation and/or the proliferation of microglia and astrocytes [6-9]. These effects are counterbalanced by those of other molecules such as IL-10, that has been shown to be neuroprotective against Glu-induced or hypoxic-ischaemic neuronal death [10], or SOCS3 (Suppressor Of Cytokine Stimulating-3). The latter acts as a negative modulator of inflammatory cytokine signalling, including that supported by IL-6 known to increase SOCS3 gene transcription [11-13]. SOCS3 mRNA up-regulation can thus be viewed as a marker of the synthesis of such cytokines [13]. Other important players are the adhesion molecules such as ICAM-1 or VCAM-1 and chemokines like KC or RANTES (*Regulates upon Activation in Normal T cells Expressed and Secreted*) that support the interaction between leukocytes and cerebral vascular endothelium, thus facilitating the penetration of immune cells from the peripheral circulation towards the CNS [14]. In the brain of rodents intoxicated with a convulsive dose of soman, a cellular inflammatory response has been described in the form of an astrocytic and microglial activation lasting for several days and being the most intense between 3 (microglia) and 7 (astroglia) days post-poisoning [15-18]. Some data also suggest a peripheral recruitment of immune cells, a phenomenon of unknown quantitative importance [16, 18]. Other recent studies, restricted to the most acute phase of the intoxication, have shown an early and transitory induction (<48h) of several neuro-inflammatory genes and IL-1β protein [19-21]. Owing to the known relationship between the cellular (glial) and molecular components of neuro-inflammation, the transient gene activation described by these authors appears rather surprising.

NA-induced SE becomes rapidly refractory to most of the treatment usually considered and especially to benzodiazepines [22], a property also shared by other types of refractory SE [23]. Antagonists of the NMDA receptors are currently the only drugs able to arrest experimental seizures and provide neuroprotection when treatment is initiated up to one hour after poisoning and beginning of seizures [24, 25]. Ketamine (KET) is one of the very few NMDA antagonists in clinical use. We recently reported that either racemic ketamine (KET), or the more active isomer S(+) KET, in combination with atropine sulphate (AS) could counteract lethality, seizures and SRBD in soman poisoned guinea-pigs even if the treatment initiation was delayed [26, 27]. Reasons for KET efficacy are probably multiple. Efficacy might be based on beneficial effects on cardiovascular or respiratory systems, or because of the NMDA

receptor blockade that can stop seizures and be neuroprotective. A NMDA-based primary mechanism was suggested by the results we obtained with S(+) KET. Neuro-inflammation being potentially of great importance for SRBD and epileptogenesis [2, 28, 29], it can also be hypothesized that KET possesses regulating effects on the excessive soman-induced neuro-inflammation through different mechanisms. Seizure control may be one of them. Indeed, using midazolam, the role of seizures in NA-induced CNS inflammation has been suggested by Chapman et al. [30]. However one cannot bring any definite proof using benzodiazepines because they also act on microglial peripheral benzodiazepine receptors [31]. Interestingly, KET also possesses anti-inflammatory properties, that cannot be related to its anti-epileptic efficacy, in various immune cells such as macrophages and other peripheral leukocytes [32, 33], but also in astrocytes and microglial cells stimulated with lipopolysaccharide (LPS) *in vitro* and *in vivo* [34].

For all these reasons, we initiated a work in two phases. First, we undertook a quantitative RT-PCR analysis of the genic response up to 7 days after intoxication in a mouse model of severe soman poisoning for which neuropathy has recently been described [16]. This time frame covers the onset of the degenerating processes (the first hours), the main phase of lesion maturation and cell death (24-48h) and the delayed period within which the most intense glial reaction can be detected [16, 17]. Following Williams et al. [19] we have quantified the changes in mRNA levels of IL-1β, TNFα, IL-6, and ICAM-1. In addition, we looked at SOCS3 mRNA. These results are currently in press [35]. Second, to evaluate the effects of KET on soman-induced neuro-inflammation, we tested two regimens of racemic KET combined with AS (10 mg/kg, i.p.), first administration of which started either 30 min or 60 min post-toxic exposure. In these conditions and at one set time point only (48h), we performed a quantitative analysis of the genic response (IL-1β, TNFα, IL-6, ICAM-1, SOCS3), and corresponding protein synthesis (except for SOCS3). KC, RANTES and IL-10 protein expression was also studied. We focused on the hippocampus and the cortex, two structures known to be injured during soman-induced seizures.

2.0 MATERIELS & METHODS

Soman, >97% pure by gas chromatography, was supplied by the Centre d'Etudes du Bouchet (Vert-Le-Petit, France). The oxime HI-6 (1-2-hydroxy-iminomethyl-1-pyridino-2-oxanopropane) dichloride was a generous gift of DRDC Suffield (Canada). Atropine sulphate (AS) was from Sigma Chemicals (L'Isle d'Abeau Chesnes, France). Ketamine hydrochloride, KET (Ketamine Panpharma®; 5% solution as hydrochloride, Panpharma, Fougères, France) as well as other drugs whenever necessary were diluted in sterile saline immediately prior to their use.

2.1. Animals

Adult male Swiss mice (ca. 30 g, Janvier, France) served as subjects. The animals were housed on a 12 h dark/light cycle with light provided between 7 a.m. and 7 p.m. They were given food and water *ad libitum*. All experiments were approved by our Institutional Animal Care and Research Advisory Committee in accordance with the applicable French and European community regulations. Animals were randomly distributed across the various groups.

2.2 Pre-treatment and poisoning

The day of the experiment, mice that were poisoned received an injection of HI-6 (50 mg/kg in saline; 200µl) 5 min prior to the administration of a convulsive dose of soman (172 µg/kg; 0.6 LD_{50} in the presence of HI-6; 1.6 LD50 in the absence of HI-6; 200µl). Appropriate control groups were constituted, substituting saline to HI-6 or soman (see below). Except for soman that was injected subcutaneously, all drugs were given i.p. This model of intoxication is known to produce convulsive EEG seizures [36], significant neuropathy and good survival in almost all the animals [16]. In absence of EEG in our experimental setting, the clinical state, including the development of convulsions, was continuously

observed for ca. 3 h after soman intoxication and then every hour until the end of the day. All poisoned mice included in the present work showed long-lasting convulsions indicative of development of seizures. For the assessment of KET/AS treatment, animals that did not convulse or demonstrated limited motor signs before KET/AS were excluded from further treatment.

2.3. Soman-induced transcription of selected genes

2.3.1. Time-course study

Following exposure to soman (To), animals were decapitated at set time points, *viz* To + 30 min, 1 h, 2 h, 6 h, 24 h, 48 h and at day (d) 7. Two control groups that were not poisoned but received instead an injection of saline were constituted. They differed by the administration of HI-6 in the "HI-6 control group" and saline in the "no-HI-6 control group". They were only sampled at 6 h and 24 h post-injection because published work [19] and our pilot experiments (data not shown) both showed a peak of mRNA induction between 6h and 24 h post-soman, depending on the genes studied.

2.3.2. Effects of KET/AS

Two groups were constituted, each receiving one of the regimens of racemic KET combined with AS (10 mg/kg). The first group (Soman KET25) received six injections of AS, combined with 25 mg/kg KET every 30 min, starting 30 minutes post-poisoning (Sub-Anaesthetic Protocol). The second group (Soman KET100) received three injections of AS, combined with 100 mg/kg KET, every 60 min, the first injection being delayed by one hour post-poisoning (Anaesthetic Protocol).

At difference with the time-course study, animals of the soman group were repeatedly treated with saline/AS (total of 6 injections) in a protocol matching that of the soman KET25 group. AS might indeed have an effect *per se* [37]. For this study, two control groups were constituted. They were both treated with HI-6, before receiving saline instead of soman and KET/AS treatment. They matched the two KET experimental groups (KET25 control group and KET100 control group). Animals were decapitated 48 h post intoxication.

2.3.3. mRNA quantification

After dissection on ice, brain structures (whole cortex and hippocampus) were immediately placed in 1 ml RNALater® (Ambion, Austin, USA) and kept at +4°C for 24 h. Then samples were stored at -20°C until RNA extraction. For RNA isolation, samples were thawed on ice, and isolation was carried out using RNA InstaPur® (Eurogentec, Saraing, Belgium) according to the manufacturer's instructions. Reverse transcription was performed in a 20 µl final volume containing 1.5 µg RNA, using the Reverse Transcriptase Core Kit® (Eurogentec, Saraing, Belgium) with 500 µM of each oligo d(T) and ribonuclease inhibitor (80 U) according to the manufacturer's instructions. Oligonucleotide primers were synthesized at Eurogentec (Saraing, Belgium). The primer design and optimization regarding the primer dimmer, the self-priming formation and the primer melting temperature were done with MacVector® software (Accelrys, San Diego, USA). Specificities of the PCR amplification were documented with LightCycler® melting curve analysis. Amplification products obtained were controlled by high performance gel electrophoresis with DNA Lab Chips® (Agilent technologies, USA). Melting peaks obtained either from RT-product or from specific recombinant DNA were identical. The selected forward (F) and reverse (R) primer sequences are listed in Table 1.

Table 1 : Sequences of Forward (F) and Reverse (R) primers used for quantitative PCR. HPRT: hypoxanthine phosphoribosyl transferase; CycA: cyclophilin A; ARBP: acidic ribosomal phosphoprotein ; TBP: TATA box Binding Protein

Gene	Accession Number	5'-3' sequence	Product size (bp)	Annealing temperature (°C)
HPRT	NM_013556	F CTCATGGACTGATTATGGACAGGAC R GCAGGTCAGCAAAGAACTTATAGCC	123	60
CycA	NM_009828	F CATCTGCACTGCCAAGACTGAATG R CTTCTTGCTGGTCTTGCCATTCC	127	58
ARBP	NM_007475	F GAAAATCTCCAGAGGCACCATTG R TCCCACCTTGTCTCCAGTCTTTATC	70	54
TBP	NM_011603	F CGGACAACTGCGTTGATTTTCAG R GAAGCCCAACTTCTGCACAACTC	117	56
IL-1β	NM_008361	F GCTGAAAGCTCTCCACCTCAATG R TGTCGTTGCTTGGTTCTCCTTG	88	58
IL-6	NM_031168	F GAGGATACCACTCCCAACAGACC R AAGTGCATCATCGTTGTTCATACA	141	60
TNFα	NM_013693	F CATCTTCTCAAAATTCGAGTGACAA R TGGGAGTAGACAAGGTACAACCC	175	60
SOCS3	NM_007707	F CCAGCTCCAGCTTCTTTCAAGTG R GAGAGTCCGCTTGTCAAAGGTATTG	73	60
ICAM-1	NM_010493	F GGGCTGGCATTGTTCTCTAATGTC R GGATGGTAGCTGGAAGATCGAAAG	69	59

The PCR was carried out with the LC Fast Start DNA Master SYBR Green® kit (Roche Applied Science, Mannheim, Germany) using 5 µl of cDNA (equivalent to 40 ng of total RNA) in a final volume of 20 µl, 4 mM $MgCl_2$ and 0.4 µM of each primer (final concentration). The quantitative PCR were performed using a Lightcycler® (Roche Applied Science, Mannheim, Germany) for 45 cycles at 95°C for 20 s (denaturation), 54-60°C for 5 s (annealing temperature, which is primer dependent, Table 1), and a final step of 10 s at 72°C (elongation). Crossing point values were calculated from Lightcycler® Software v.3.5 (Roche Applied Science, Mannheim, Germany) using the second derivative maximum method.

Quantification was achieved using a pool of all the cDNA samples as calibrator [38] according to the comparative threshold cycle method [39]. The relative mRNA values were calculated with the RealQuant® software (Roche Applied Science). For the time course study, we originally used a single internal control gene and mRNA levels were normalized to the corresponding level of HPRT, a commonly used housekeeping gene, which expression does not appear to be significantly altered by seizures (data not shown). To improve quantification in the second study, normalization was performed by using the geometric average of several validated internal control genes: CycA, HPRT, TBP and ARBP for the cortex and CycA, HPRT and TBP for the hippocampus. Expression stability of the potential reference genes was assessed using GENORM software [40].

2.4. Effects of soman on selected proteins and modification by KET/AS

Five out of the seven treatment groups that were used are identical to those previously described in the study of KET/AS effects on soman-induced inflammatory gene transcription. In order to evaluate the potential effects of KET on protein levels, a control group receiving no KET matched the KET100 control group. Finally, HI-6 injection was even omitted in the last control group.

Animals were decapitated 48 h post intoxication. After dissection on ice, brain structures (whole cortex and hippocampus) were isolated and frozen on dry ice and stored at -80°C. For analysis, hippocampal and whole cortex samples were respectively thawed in 0.5 ml and 1.0 ml of cell lysis buffer (Cell Lysis Kit #171-304012, Bio-Rad Life Science Group, Marnes-la-Coquette, France) containing a protease inhibitor cocktail (#171-304012; Bio-Rad Life Science Group) and 3 µl of a stock solution containing 500 mM phenylmethylsulfonyl fluoride (#P-7626) in dimethyl sulphoxide (#D2650), both from Sigma (L'Isle d'Abeau Chesnes, France). Then, samples were homogenized with a high-speed Polytron PT3100 (Fischer Scientific, Illkirch, France) at +4°C using four rapid pulses. Samples were then centrifuged at 4500×*g* for 15 min at +4°C, supernatants collected and stored at -80°C. Total protein concentration was determined using the BCA protein Assay kit (#23225 Pierce; Interchim, Illkirch, France). All tissue samples were diluted with the cell lysis buffer to a final total protein concentration of 1000 µg/ml. Tissue homogenates were assayed for cytokines, chemokines and adhesion molecules using multiplexed bead-based immunoassay kits for IL-1β, IL-6, TNFα, IL-10, KC and RANTES (Bio-Rad Life Science Group) and ICAM-1, VCAM-1 (Millipore Linco Biosciences Division, Saint Quentin en Yvelines, France), according to the manufacturers' instructions.

2.5. Statistical analysis

When multiple groups were compared, a non-parametric procedure was followed: either Mann-Whitney test (2 groups) or a Kruskal-Wallis ANOVA by ranks followed by post-hoc pairwise comparison with the Mann-Whitney test with Bonferroni correction. Analysis was performed in two steps. First difference between control groups was tested and if not statistically different they were pooled and constituted a single group incorporated in the relevant comparisons with other experimental groups.

3. RESULTS

3.1. Soman-induced transcription of selected genes

Whatever the time-point (T0 + 6 h or + 24 h) and the mRNA considered, no significant difference in mRNA levels was ever detected between the no-HI-6 control group and the HI-6 control group. As the use of HI-6 did not seem to influence the neuro-inflammatory gene response in our experimental conditions, all the controls were pooled for further analysis.

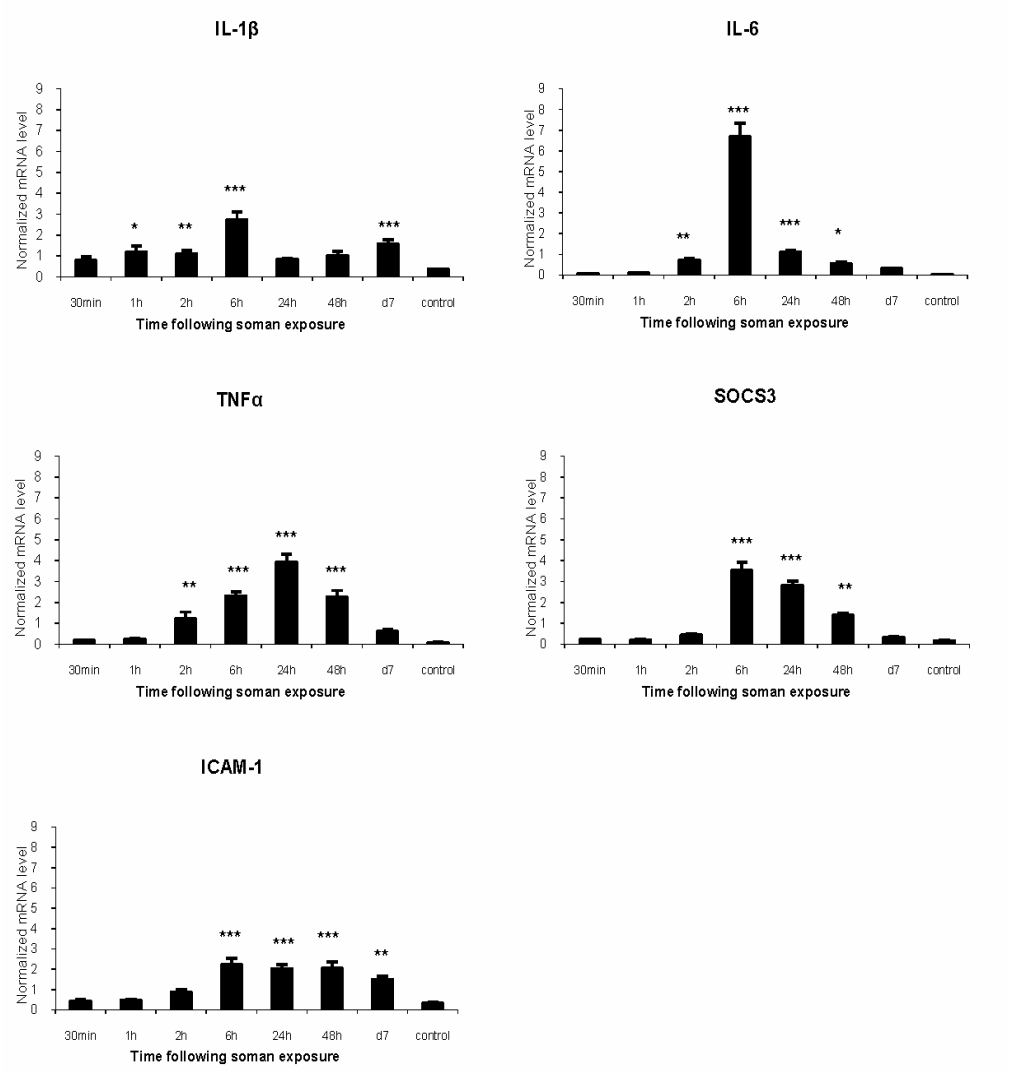

Fig. 1. Quantitative RT-PCR analysis of changes in inflammatory gene expression following soman intoxication in mouse cortex. Data are presented as the mean ± SEM. mRNA levels were normalized to the corresponding level of HPRT (n=5-7/group). Only the statistically significant comparisons to the combined control group (HI-6 controls +no-HI-6 controls) are represented. (*) α<0,05 (**) α<0,01 (***) α <0,001. From [35].

In response to soman intoxication, an important and highly significant increase in mRNA levels was recorded in cortex (Fig. 1) and hippocampus (Fig. 2) for the five neuro-inflammatory genes studied. At their peak, the values could be as high as 151-fold those measured in the controls. Interestingly the gene response in the cortex generally appeared sooner compared to the hippocampus: the first significant increase was 1-6 h and 6 h and the peak response was 6-24 h and 24-48 h, for cortex and hippocampus respectively. After the peak period, there was a trend towards a decrease of the mRNA levels. However, mRNA were not back to control level at d7 and remained significantly elevated for two genes (IL-1β, ICAM-1) in the cortex and for four out of the five genes (IL-1β, TNFα, SOCS3, ICAM-1) in the hippocampus. In the cortex, the response of the IL-1β gene seems biphasic: the peak response observed at 6 h was followed at 24 h and 48 h by a decrease without normalization, although the difference to control level did not reach significance, and then by a significant re-increase at d7 (figure 1).

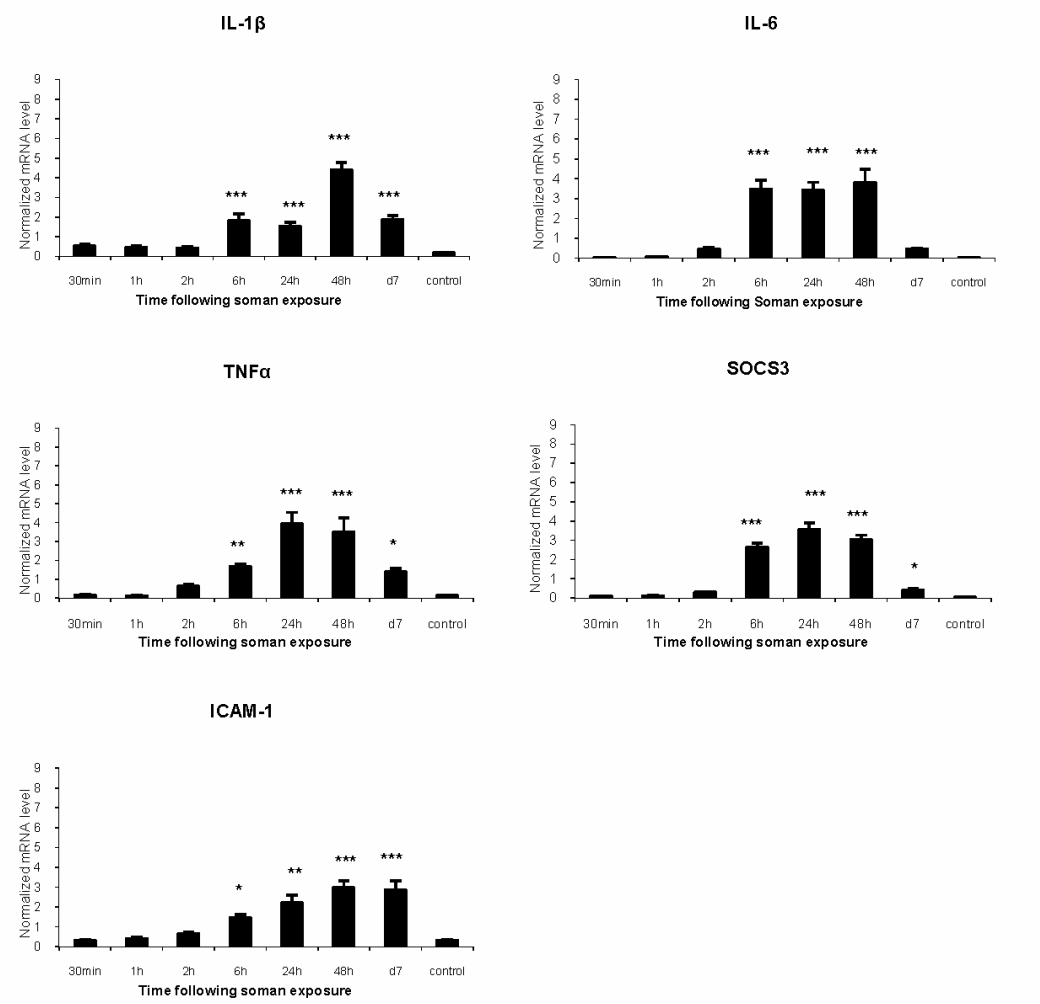

Fig. 2. Quantitative RT-PCR analysis of changes in inflammatory gene expression following soman intoxication in mouse hippocampus. Data are presented as the mean ± SEM. mRNA levels were normalized to the corresponding level of HPRT (n=5-7/group). Only the statistically significant comparisons to the combined control group (HI-6 controls +no-HI-6 controls) are represented. (*) α<0,05 (**) α<0,01 (***) α <0,001. From [35].

3.2. Treatment of soman poisoning with KET/AS

None of the control groups used in the mRNA or protein study significantly differed from each other. Hence, they were all pooled for further analysis. The repeated administration of AS (10 mg/kg) to soman-poisoned animals did not prevent the large and significant increase in mRNA from the selected genes observed 48 h after challenge (Fig. 3, 4 and 5).

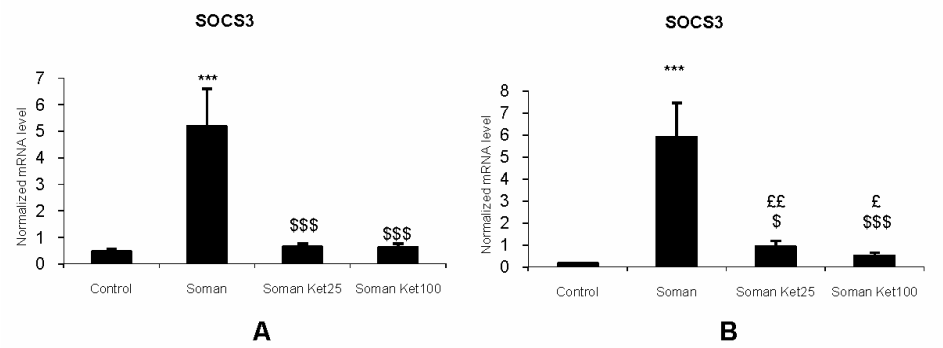

Fig. 3. Quantitative RT-PCR analysis of changes in SOCS3 gene expression following soman intoxication, and KET/AS treatment with sub-anaesthetic (Soman-KET25) or anaesthetic (Soman-KET100) protocol in mouse cortex (A) and hippocampus (B). Data are presented as the mean ± SEM. mRNA levels were normalized to the geometric average of CycA, HPRT and TBP levels (as well as ARBP for cortex) (n=5-14/group). The statistical comparisons between the different groups and the combined control-group are represented by *. Each of the KET-treated groups was statistically compared either to the soman group ($) or to the combined control-group (£). The combined control-group consists of KET25 control group and KET100 control group as defined in Material and methods. (*,$,£) α<0,05 (,$$,££) α<0,01 (***,$$$,£££) α <0,001**

An early treatment with KET25/AS was able to significantly decrease mRNA levels both in cortex and hippocampus, 48 h post-soman exposure although normalization was not always achieved. This was particularly prominent for SOCS3 and TNFα mRNA both in cortex (Fig. 3, Fig. 4) and hippocampus (Fig. 3, Fig. 5) as well as for IL-6 mRNA in hippocampus. Il-1β mRNA in whole cortex was a notable exception (Fig. 4).

The mRNA induction detected after soman intoxication was usually followed by an increase of the related proteins at the time of the experiment, 48 h (Fig. 4, 5, 6). TNFα and IL-6 showed a moderate increase that did not reach statistical significance in whole cortex (Fig. 4) at difference with hippocampus (Fig. 5). Chemokines (KC, RANTES) and adhesion molecule ICAM-1 were clearly increased after soman intoxication, both in cortex and hippocampus (Fig. 6). Conversely, VCAM-1 was changed neither in the cortex, nor in the hippocampus (data not shown). Interestingly, soman also induced a decrease in the anti-inflammatory IL-10 that could be quantified in hippocampus. In whole cortex, for any of the experimental groups, IL-10 was below the limit of detection (Fig. 6). When given 30 min post soman exposure, the AS and KET (25 mg/kg) combination had a clear effect on the selected proteins, assayed 48h after challenge. With exception of TNFα, other pro-inflammatory mediators always tended to be decreased although differences existed depending on the mediator and structure analysed (Fig. 4, 5, 6). The global anti-inflammatory effect of the combination was also supported by a return to control levels of IL-10.

When the treatment was delayed, but the dose of KET increased to 100 mg/kg, comparable beneficial effects could be observed either on mRNA or protein levels (Fig. 3, 4, 5, 6).

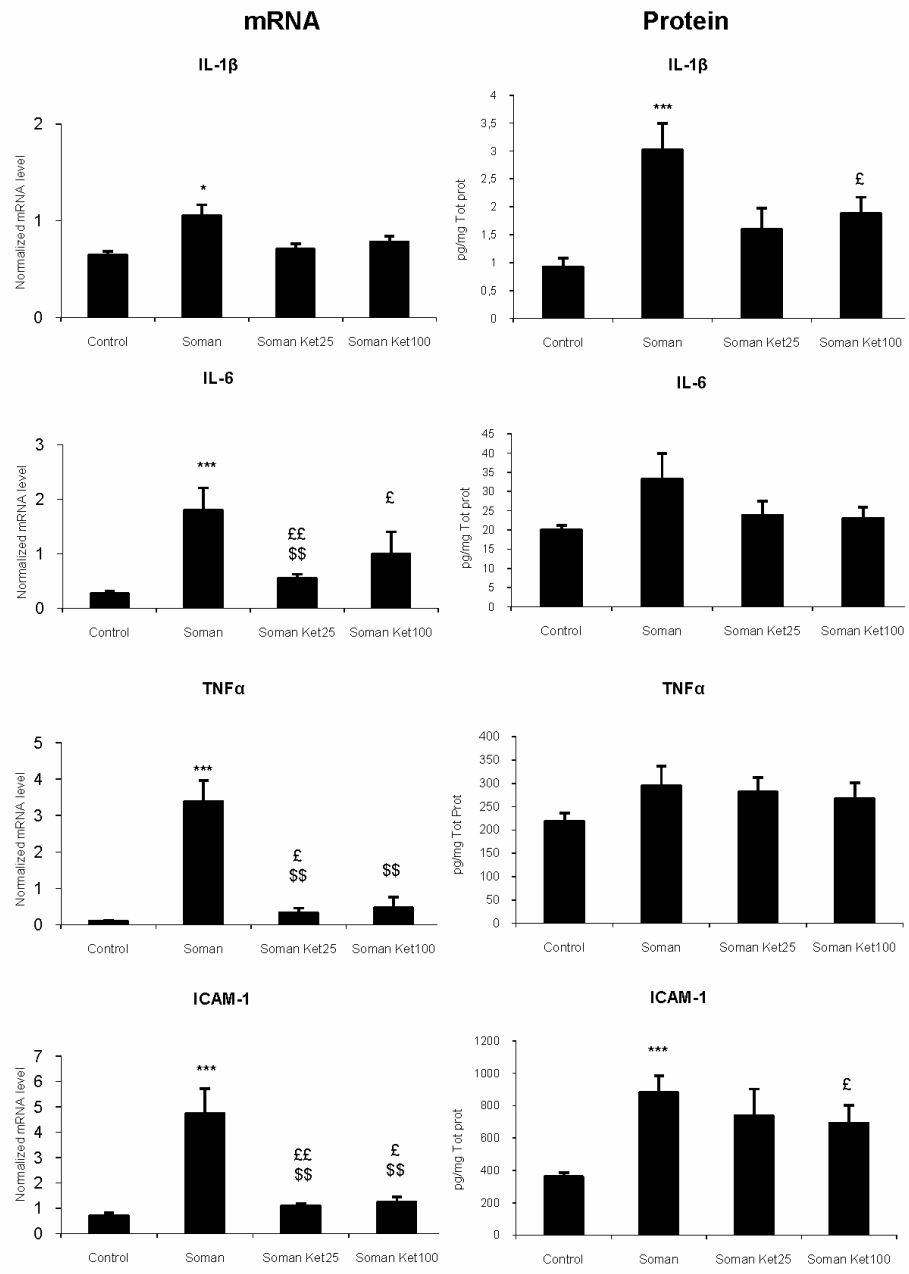

Fig. 4. Analysis in cortex by quantitative RT-PCR (left column) and by multiplexed bead-based protein immunoassays (right column) of soman-induced changes in inflammatory cytokines, chemokines and adhesion molecules mRNA and protein respectively and their modification after KET/AS administration. Two protocols of KET/AS administration were studied as described in Material and methods. Data are presented as the mean ± SEM. mRNA levels were normalized to the geometric average of CycA, HPRT, ARBP and TBP levels (n=5-14/group).The statistical comparisons between the soman group and the combined control-group are represented by *. Each of the KET-treated groups was statistically compared either to the soman group ($) or to the combined control-group (£). The combined control-group consists of KET25 control group and KET100 control group and two other control groups matching the latter but without KET and with or without HI-6. (*,$,£) α<0,05 (,$$,££) α<0,01 (***,$$$,£££) α <0,001**

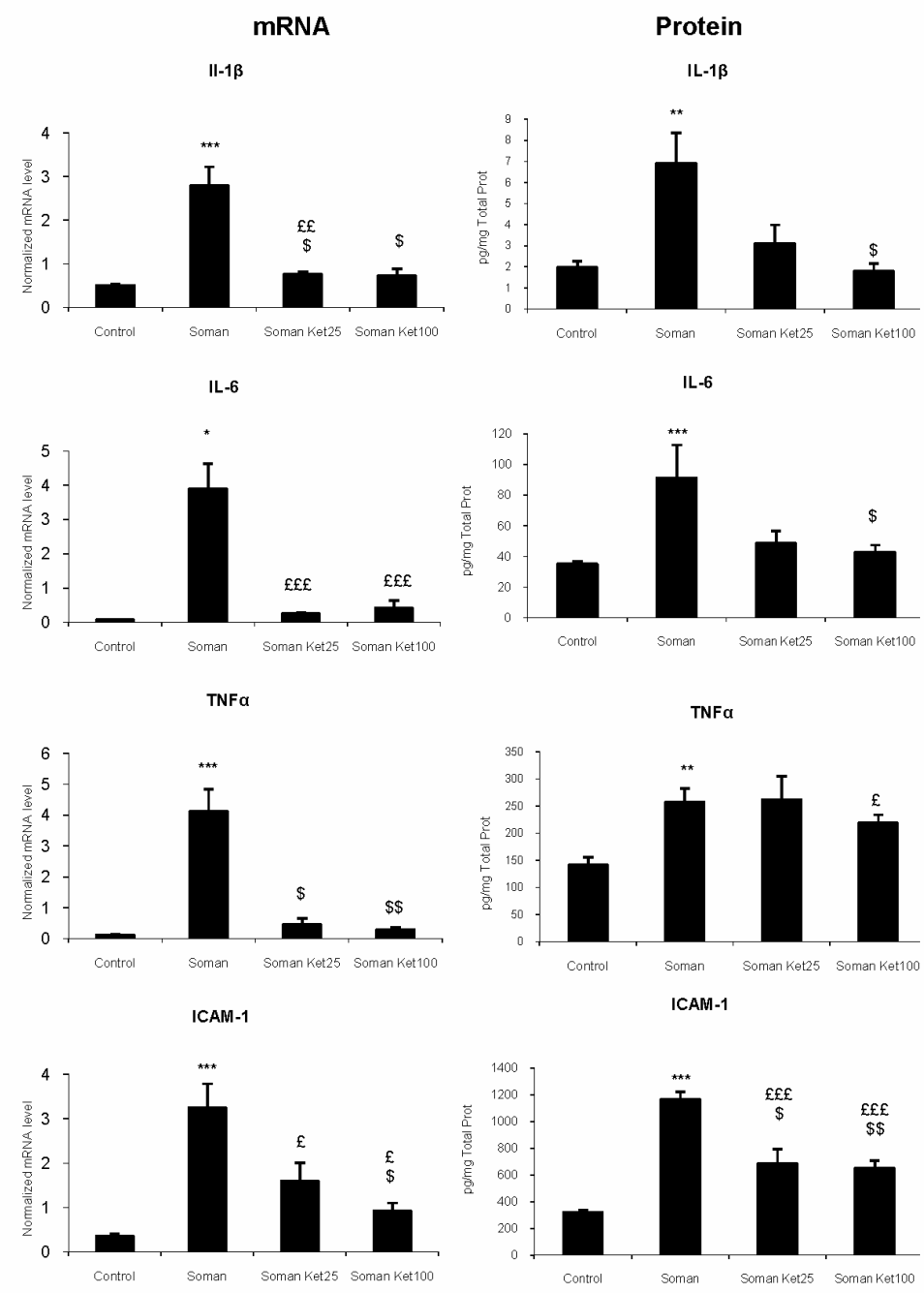

Fig. 5. Analysis in hippocampus by quantitative RT-PCR (left column) and by multiplexed bead-based protein immunoassays (right column) of soman-induced changes in inflammatory cytokines, chemokines and adhesion molecules mRNA and protein respectively and their modification after KET/AS administration. Two protocols of KET/AS administration were studied as described in Material and methods. Data are presented as the mean ± SEM. mRNA levels were normalized to the geometric average of CycA, HPRT and TBP levels (n=5-14/group).The statistical comparisons between the soman group and the combined control-group are represented by *. Each of the KET-treated groups was statistically compared either to the soman group ($) or to the combined control-group (£). The combined control-group consists of KET25 control group and KET100 control group and two other control groups matching the latter but without KET and with or without HI-6. (*,$,£) α<0,05 (,$$,££) α<0,01 (***,$$$,£££) α <0,001**

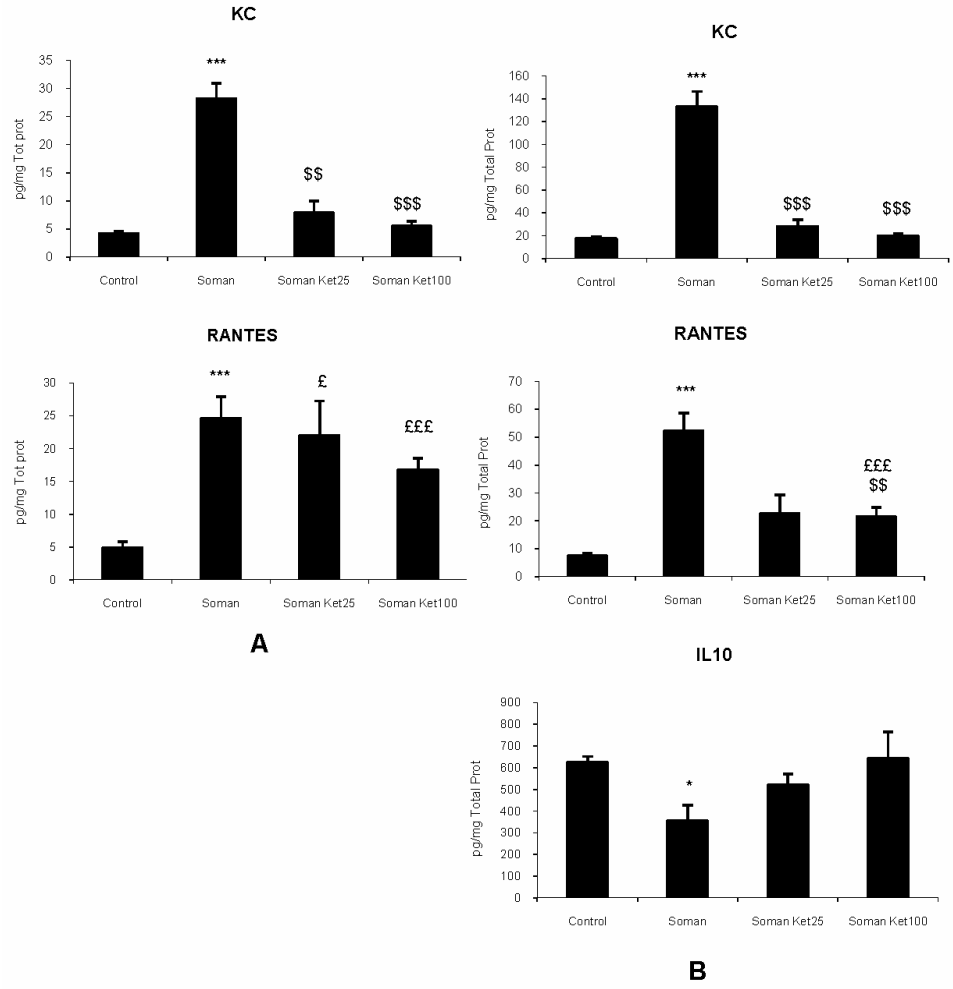

Fig. 6. Analysis by multiplexed bead-based protein immunoassays in cortex (A) and in hippocampus (B) of soman-induced changes in chemokines and IL-10 and their modification after KET/AS administration. Two protocols of KET/AS administration were studied as described in Material and methods. In cortex, IL-10 level was below the detection limit in our experimental conditions. Data are presented as the mean ± SEM (n=5-10/group).The statistical comparisons between the soman group and the combined control-group are represented by *. Each of the KET-treated groups was statistically compared either to soman group ($) or to the combined control-group (£). The combined control-group consists of KET25 control group and KET100 control group and two other control groups matching the latter but without KET and with or without HI-6. (*,$,£) α<0,05 (,$$,££) α<0,01 (***,$$$,£££) α <0,001**

4. DISCUSSION

Following a variety of neurotoxic insults, active brain inflammation is promoted by the production of different kind of molecules such as cytokines. These can be produced by several types of cells including neurons, microglia, astrocytes as well as leukocytes entering the brain [41]. Cytokines have a wide range of biological activities and can participate both in neurotoxic and repair processes [3, 42]. Neuro-inflammatory response has been described in different models of chemically-induced seizures such as those produced by kainate [29] and lithium-pilocarpine [43] as well as by organophosphorus NA such as sarin [30] and soman [19-21]. Rodents exposed to high doses of soman also quickly develop convulsive seizures and accompanying neuropathy in various brain structures including the hippocampus and the

cortex [44-46]. More specifically, mice poisoned according to our protocol were previously reported to show brain damage that includes progression of oedema [36], development of different types of cell death as well as reactive gliosis that participate in inflammatory events up to 7 days after poisoning [16].

In our model of severe seizures and SRBD, we showed here that the induced transcription of genes of special importance in neuro-inflammation has a relatively quick onset and is long-lasting. Indeed, it did not wane even 7 days after poisoning. This may be related to the time course of the glial response to poisoning. An astrocytic reactivity in the form of cell swelling [16, 47] and stimulation of the Glial Fibrillary Associated Protein gene [16, 18] was reported as early as the first hours of soman-induced seizures. However, the most massive hyperplasic astrocytic response was consistently found from 3 to 8 days after intoxication [16, 17]. An extensive microglial reaction was also observed in different models. It seems to appear in the first hours of soman-induced convulsions, [18], to peak three days after the intoxication [17] and to be still massive at d7 [16]. With the purpose to establish firmer relationships between the glial and neuro-inflammatory reactions, it will be of high interest to complement our work by a more thorough comparison between the kinetics of gene and protein expression and that of brain damage, microglial and astrocytic activation. It may be especially important to include time-points between d2 and d7, and later. This might reveal multiphasic gene responses. Indeed, the biphasic response we obtained in the cortex with IL-1β may be suggestive of such a phenomenon. Our results also demonstrate differences between hippocampus and cortex for the time course of neuro-inflammatory gene transcription. mRNA levels began to rise and peaked sooner in the cortex. One possible explanation is that piriform cortex, and probably parts of the amygdalian complex, could have been included in our whole cortex samples. These two structures are described as particularly sensitive to the seizurogenic and neuropathological effects of soman [37] (and references therein). Indeed, piriform cortex is known to be involved during the initiation of soman-induced seizures and to react very rapidly to the poisoning by early gene expressions [48, 49] as well as structural and molecular changes in neurons and glial cells [18, 50]. In contrast, the hippocampus is rather a “target“ area to which the paroxysmal inputs could propagate from the amygdala and piriform cortex [46] and in which the cellular changes are less prevalent and the easiest to counteract by effective anticonvulsants [48, 51, 52]. Nonetheless, in a rat model of soman poisoning, no such difference between hippocampus and piriform cortex was described by Williams et al. [19]. This difference in cortex sample composition may also have partly accounted for the quantitative differences in the gene response between our study and that of Williams et al. [19]. Finally, it has also probably impacted on the protein levels and a mixture of damaged and less damaged structures in our samples would have caused a dilution. It may explain why, except for TNFα, hippocampus samples always showed higher content in the specific proteins studied.

By studying the protein levels 48 h post soman-exposure, we also showed here that for the tested factors, mRNA increase was followed by protein synthesis. This suggests that, in our conditions, seizures did not heavily impact on protein translation as opposed to previously published data [53, 54]. However, the soman-induced changes in specific protein contents were not always as important as modifications in mRNA levels. This was particularly prominent for TNFα. A mismatch between mRNA and protein levels is not uncommon owing to the different types of post-transcriptional and translational regulations. Interestingly, such regulations are known to occur for TNFα [55-58].

Brain inflammation is not only supported by resident cells but also by the recruitment of blood-borne cells. Some cytokines play an active role by increasing the expression of chemokines and cell adhesion molecules (CAMs) [14]. This has been described not only in pathological brain conditions [59-61] but also in normal hippocampus, cortex and thalamus [62]. In the case of soman-induced seizures and SRBD, the increase in mRNA for ICAM-1 (our study and Williams et al. [19]), VCAM-1 and E-selectin [19] and the increase in ICAM-1, KC and RANTES (our study) support such a phenomenon. The absence of significant changes in VCAM levels in our model cannot be discussed in absence of data on transcription. Zimmer et al. [49] observed numerous pictures of macrophage-like cells during the first 8 h of soman poisoning, but this cannot be viewed as a definite proof of migration of blood-borne

macrophages into the brain owing to the present difficulty to differentiate them from resident reactive microglial cells. Nonetheless, a probable invasion of the brain by blood cells is supported by a recent ultrastructural study that revealed numerous polymorphonuclear macrophagic cells 7 days after the intoxication [16]. A quantitative assessment of these events in soman poisoning is still lacking and should be considered to get a broader picture of soman-induced neuro-inflammation.

Not only soman does promote an important increase in pro-inflammatory factors but it also reduces the protective molecules as shown by a significant decrease in IL-10 in hippocampus. IL-10 decreases the vulnerability of neurons after ischaemia and trauma [63, 64] and inhibits the synthesis of inflammatory cytokines, including TNF-α and IL-1β [64, 65]. The soman-induced decrease is not in the line of other data showing that microglial cells produce IL-10 after LPS stimulation [66, 67] and that IL-10 is highly increased in cerebrospinal fluid and serum of stroke patients [68, 69]. It would thus be important to understand the mechanisms involved to possibly counteract the effects of soman on anti-inflammatory physiological messengers.

In the time course study, we could not show any effect of HI-6 on the transcription of neuro-inflammatory markers in the brain areas tested. Our paradigm cannot allow us to compare our results with those previously reported by Svensson et al. [21] that show, 30 min after the injection of HI-6, a transient increase of IL-1β protein. This effect was detected only in the frontal cortex of rats and was no longer detectable 6 hours after injection, our first set time for mRNA analysis. Addition of any drug in experimental paradigms can always confound results: HI-6 in our study, atropine methylnitrate (AMN) + HI-6 in that of Williams et al. [19]. In this line of thought, it is interesting to report that in another mouse model, the use of AMN as an immediate post-treatment decreased the leukocyte count, including the granulocytes, and thus may have an impact on inflammation (Collombet et al., unpublished results, personal communication). However, in our experiments, repeated injections of AS in non-poisoned individuals did not significantly modify any of the proteins studied.

Conversely, adjunction of KET to AS induced a general tendency to reverse soman effects whatever the mRNA or protein levels considered. However, for most of the parameters, levels were not back to control levels. Although AS may contribute to the anti-inflammatory effect by acting on glial cells [70], it does not prevent soman-induced brain inflammation (our soman control group received AS alone). More specifically, in hippocampus, both KET/AS regimens were highly efficient in reducing mRNA levels for SOCS3, IL-1β, IL-6 and TNFα but the impact on IL-1β and IL-6 proteins was more limited. Conversely, TNFα protein levels were not changed suggesting that inhibition of this pathway might not be important in KET neuroprotective mechanism. Nevertheless, owing to the cascade of events that these molecules can produce, even a small reduction may have potent cellular consequences. Both KET/AS regimens also produced a significant reduction in the molecules that mediate the recruitment of blood-borne immune cells suggesting that this effect may be part of the anti-inflammatory action of KET. Finally, KET/AS tended to counteract the depressing effect of soman on IL-10 production.

On mRNA induction, the two different KET/AS were very similar in their effects although the early regimen (K25/AS) tended to be more active than the delayed treatment in the cortex while in the hippocampus the reverse was observed. Similarly, KET100/AS tended to be more effective in reducing protein levels in hippocampus. Whereas it is linked to the slower time course for gene expression in hippocampus or to the increased dose of KET remains to be studied.

How KET, a NMDA antagonist, can interact with neuro-inflammation remains to be determined. Anti-inflammatory action of KET have been described on a variety of immune cells [32, 33, 71] and glial cells [34]. However, the exact mechanisms, at the molecular level, are not defined and several pathways may be involved. KET can inhibit NF-κB activation [72], a key intracellular regulator for the transcription of the pro-inflammatory cytokine genes [73] that can be activated by seizures [74]. By normalizing IL-10 levels (our results), KET may also facilitate the negative post-transcriptional regulation (mRNA increased

instability) that IL-10 performs on IL-1β, TNFα and KC mRNA [75], hence possibly explaining in part our results. However, the positive effects we observed on RANTES cannot be attributed to such a mechanism [75]. Neuro-inflammation being both the cause and consequence of brain damage, KET may also act by limiting the duration of soman-induced seizures and SRBD [26, 27]. Alternatively KET may interact with microglial receptors as suggested by results obtained with another NMDA antagonist, MK-801 [70, 76]. Finally, KET being also well known to increase norepinephrine levels [77] this may constitute another way for KET to regulate neuro-inflammation [78].

In conclusion, we have found that severe soman poisoning induces an early gene expression of cytokines, chemokines and ICAM and thus confirmed in our model some of the results obtained by others [19, 20]. However, our results also provide evidence for differences with these earlier studies such as the fact that the neuro-inflammatory reaction is long lasting particularly in the hippocampus. We also show that the related proteins are also present in the brain two days after poisoning. The second main result brought by our study is that KET, in combination with AS, is able to significantly reduce the soman-induced neuro-inflammatory response in murine brain, even if the treatment is delayed. The mechanisms by which this combination exerts an effect on neuro-inflammation remain to be clearly understood.

5.0 REFERENCES

[1]. Yanagisawa, N., Morita, H., and Nakajima, T., *Sarin experiences in Japan: Acute toxicity and long-term effects.* J. Neurol. Sci., 2006. **249**(1): p. 76-85.

[2]. Vezzani, A., Moneta, D., Richichi, C., Aliprandi, M., Burrows, S.J., Ravizza, T., Perego, C., and DeSimoni, M.G., *Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis.* Epilepsia, 2002. **43**: p. 30-35.

[3]. Wang, C.X. and Shuaib, A., *Involvement of inflammatory cytokines in central nervous system injury.* Prog. Neurobiol., 2002. **67**(2): p. 161-172.

[4]. Chavarria, A. and Alcocer-Varela, J., *Is damage in central nervous system due to inflammation?* Autoimmun. Rev., 2004. **3**(4): p. 251-260.

[5]. Noda, M., Nakanishi, H., Nabekura, J., and Akaike, N., *AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia.* J. Neurosci., 2000. **20**(1): p. 251-258.

[6]. Bonni, A., Sun, Y., Nadal-Vicens, M., Bhatt, A., Frank, D.A., Rozovsky, I., Stahl, N., Yancopoulos, G.D., and Greenberg, M.E., *Regulation of gliogenesis in the central nervous system by the JAK-STAT signaling pathway.* Science, 1997. **278**(5337): p. 477-483.

[7]. Farber, K. and Kettenmann, H., *Physiology of microglial cells.* Brain Res. Rev., 2005. **48**(2): p. 133-143.

[8]. Deng, X.-H., Bertini, G., Xu, Y.-Z., Yan, Z., and Bentivoglio, M., *Cytokine-induced activation of glial cells in the mouse brain is enhanced at an advanced age.* Neuroscience, 2006. **141**(2): p. 645-661.

[9]. Selmaj, K.W., Farooq, M., Norton, W.T., Raine, C.S., and Brosnan, C.F., *Proliferation of astrocytes in vitro in response to cytokines. A primary role for tumor necrosis factor.* J. Immunol., 1990. **144**(1): p. 129-135.

[10]. Bachis, A., Colangelo, A.M., Vicini, S., Doe, P.P., De Bernardi, M.A., Brooker, G., and Mocchetti, I., *Interleukin-10 Prevents Glutamate-Mediated Cerebellar Granule Cell Death by Blocking Caspase-3-Like Activity.* J. Neurosci., 2001. **21**(9): p. 3104-3112.

[11]. Croker, B.A., Krebs, D.L., Zhang, J.-G., Wormald, S., Willson, T.A., Stanley, E.G., Robb, L., Greenhalgh, C.J., Forster, I., Clausen, B.E., Nicola, N.A., Metcalf, D., Hilton, D.J., Roberts, A.W., and Alexander, W.S., *SOCS3 negatively regulates IL-6 signaling in vivo.* Nat. Immunol., 2003. **4**(6): p. 540-545.

[12]. Fujimoto, M. and Naka, T., *Regulation of cytokine signaling by SOCS family molecules.* Trends Immunol., 2003. **24**(12): p. 659-666.

[13]. Lang, R., Pauleau, A.-L., Parganas, E., Takahashi, Y., Mages, J., Ihle, J.N., Rutschman, R., and Murray, P.J., *SOCS3 regulates the plasticity of gp130 signaling.* Nat. Immunol., 2003. **4**(6): p. 546-550.

[14]. Frijns, C.J.M. and Kappelle, L.J., *Inflammatory cell adhesion molecules in ischemic cerebrovascular disease.* J. Cereb. Circ., 2002. **33**(8): p. 2115-2122.

[15]. Baille-Le Crom, V., Collombet, J.M., Carpentier, P., Brochier, G., Burckhart, M.F., Foquin, A., Pernot-Marino, I., Rondouin, G., and Lallement, G., *Early regional changes of GFAP mRNA in rat hippocampus and dentate gyrus during soman-induced seizures.* Neuroreport, 1995. **7**(1): p. 365-9.

[16]. Baille, V., Clarke, P.G.H., Brochier, G., Dorandeu, F., Verna, J.M., Four, E., Lallement, G., and Carpentier, P., *Soman-induced convulsions: The neuropathology revisited.* Toxicology, 2005. **215**: p. 1-24.

[17]. Collombet, J.M., Four, E., Bernabe, D., Masqueliez, C., Burckhart, M.F., Baille, V., Baubichon, D., and Lallement, G., *Soman poisoning increases neural progenitor proliferation and induces long-term glial activation in mouse brain.* Toxicology, 2005. **208**(3): p. 319-334.

[18]. Zimmer, L.A., Ennis, M., and Shipley, M.T., *Soman-induced seizures rapidly activate astrocytes and microglia in discrete brain regions.* J. Comp. Neurol., 1997. **378**(4): p. 482-492.

[19]. Williams, A.J., Berti, R., Yao, C., Price, R.A., Velarde, L.C., Koplovitz, I., Schultz, S.M., Tortella, F.C., and Dave, J.R., *Central neuro-inflammatory gene response following soman exposure in the rat.* Neurosci. Lett., 2003. **349**(3): p. 147-150.

[20]. Svensson, I., Waara, L., Johansson, L., Bucht, A., and Cassel, G., *Soman-induced interleukin-1 beta mRNA and protein in rat brain.* Neurotoxicology, 2001. **22**(3): p. 355-362.

[21]. Svensson, I., Waara, L., and Cassel, G., *Effects of HI 6, Diazepam and Atropine on Soman-Induced IL-1[beta] Protein in Rat Brain.* Neurotoxicology, 2005. **26**(2): p. 173-181.

[22]. Shih, T.M., McDonough, J.H., and Koplovitz, I., *Anticonvulsants for soman-induced seizure activity.* J. Biomed. Sci., 1999. **6**(2): p. 86-96.

[23]. Chen, J.W. and Wasterlain, C.G., *Status epilepticus: pathophysiology and management in adults.* The Lancet Neurology, 2006. **5**(3): p. 246-256.

[24]. Carpentier, P., Foquin-Tarricone, A., Bodjarian, N., Rondouin, G., Lerner-Natoli, M., Kamenka, J.M., Blanchet, G., Denoyer, M., and Lallement, G., *Anticonvulsant and antilethal effects of the phencyclidine derivative TCP in soman poisoning.* Neurotoxicology, 1994. **15**(4): p. 837-51.

[25]. Lallement, G., Baubichon, D., Clarencon, D., Galonnier, M., Peoch, M., and Carpentier, P., *Review of the value of gacyclidine (GK-11) as adjuvant medication to conventional treatments of organophosphate poisoning: Primate experiments mimicking various scenarios of military or terrorist attack by soman.* Neurotoxicology, 1999. **20**(4): p. 675-684.

[26]. Dorandeu, F., Baille, V., Mikler, J., Testylier, G., Lallement, G., Sawyer, T., and Carpentier, P., *Protective effects of S(+) ketamine and atropine against lethality and brain damage during soman-induced status epilepticus in guinea-pigs.* Toxicology, 2007. **234**(3): p. 185-193.

[27]. Dorandeu, F., Carpentier, P., Baubichon, D., Four, E., Bernabe, D., Burckhart, M.F., and Lallement, G., *Efficacy of the ketamine-atropine combination in the delayed treatment of soman-induced status epilepticus.* Brain Res., 2005. **1051**(1-2): p. 164-175.

[28]. De Simoni, M.G., Perego, C., Ravizza, T., Moneta, D., Conti, M., Marchesi, F., De Luigi, A., Garattini, S., and Vezzani, A., *Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus.* Eur. J. Neurosci., 2000. **12**(7): p. 2623-2633.

[29]. Vezzani, A., Conti, M., De Luigi, A., Ravizza, T., Moneta, D., Marchesi, F., and De Simoni, M.G., *Interleukin-1beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: functional evidence for enhancement of electrographic seizures.* J. Neurosci., 1999. **19**(12): p. 5054-5065.

[30]. Chapman, S., Kadar, T., and Gilat, E., *Seizure duration following sarin exposure affects neuro-inflammatory markers in the rat brain.* Neurotoxicology, 2006. **27**(2): p. 277-283.

[31]. Wilms, H., Claasen, J., Rohl, C., Sievers, J., Deuschl, G., and Lucius, R., *Involvement of benzodiazepine receptors in neuroinflammatory and neurodegenerative diseases: evidence from activated microglial cells in vitro.* Neurobiol. Dis., 2003. **14**(3): p. 417-424.

[32]. Takenaka, I., Ogata, M., Koga, K., Matsumoto, T., and Shigematsu, A., *Ketamine suppresses endotoxin-induced tumor necrosis factor alpha production in mice.* Anesthesiology, 1994. **80**(2): p. 402-408.

[33]. Kawasaki, T., Ogata, M., Kawasaki, C., Ogata, J., Inoue, Y., and Shigematsu, A., *Ketamine suppresses proinflammatory cytokine production in human whole blood in vitro.* Anesth. Analg., 1999. **89**(3): p. 665-669.

[34]. Shibakawa, Y., Sasaki, Y., Goshima, Y., Echigo, N., Kamiya, Y., Kurahashi, K., Yamada, Y., and Andoh, T., *Effects of ketamine and propofol on inflammatory responses of primary glial cell cultures stimulated with lipopolysaccharide.* Br. J. Anaesth., 2005. **95**: p. 803-810.

[35]. Dhote, F., Peinnequin, A., Carpentier, P., Baille, V., Delacour, C., Foquin, A., Lallement, G., and Dorandeu, F., *Prolonged inflammatory gene response following soman-induced seizures in mice.* Toxicology, 2007. **in press**.

[36]. Testylier, G., Lahrech, H., Montigon, O., Foquin, A., Delacour, C., Bernabe, D., Segebarth, C., Dorandeu, F., and Carpentier, P., *Cerebral edema induced in mice by a convulsive dose of soman. Evaluation through diffusion-weighted magnetic resonance imaging and histology.* Toxicol. Appl. Pharm., 2007. **220**(2): p. 125-137.

[37]. Carpentier, P., Foquin, A., Rondouin, G., Lerner-Natoli, M., de Groot, D.M.G., and Lallement, G., *Effects of atropine sulphate on seizure activity and brain damage produced by soman in guinea-pigs: ECoG correlates of neuropathology.* Neurotoxicology, 2000. **21**(4): p. 521-540.

[38]. Peinnequin, A., Mouret, C., Birot, O., Alonso, A., Mathieu, J., Clarencon, D., Agay, D., Chancerelle, Y., and Multon, E., *Rat pro-inflammatory cytokine and cytokine related mRNA quantification by real-time polymerase chain reaction using SYBR green.* BMC Immunol., 2004. **5**(3): p. 1471-1480.

[39]. Livak, K.J. and Schmittgen, T.D., *Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-[Delta][Delta]CT Method.* Methods, 2001. **25**(4): p. 402-408.

[40]. Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., and Speleman, F., *Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes.* Genome Biol., 2002. **3**(7): p. research0034.1 - research0034.11.

[41]. Viviani, B., Bartesaghi, S., Corsini, E., Galli, C.L., and Marinovich, M., *Cytokines role in neurodegenerative events.* Toxicol. Lett., 2004. **149**: p. 85-89.

[42]. Allan, S.M. and Rothwell, N.J., *Cytokines and acute neurodegeneration.* Nat. Rev. Neurosci., 2001. **2**(10): p. 734-744.

[43]. Voutsinos-Porche, B., Koning, E., Kaplan, H., Ferrandon, A., Guenounou, M., Nehlig, A., and Motte, J., *Temporal patterns of the cerebral inflammatory response in the rat lithium-pilocarpine model of temporal lobe epilepsy.* Neurobiol. Dis., 2004. **17**(3): p. 385-402.

[44]. Carpentier, P., Lambrinidis, M., and Blanchet, G., *Early dendritic changes in hippocampal pyramidal neurones (field CA1) of rats subjected to acute soman intoxication: a light microscopic study [published erratum appears in Brain Res. 1991 May 31;350(1):179].* Brain Res., 1991. **541**(2): p. 293-9.

[45]. Lemercier, G., Carpentier, P., Sentenac-Roumanou, H., and Morelis, P., *Histological and histochemical changes in the central nervous system of the rat poisoned by an irreversible anticholinesterase organophosphorus compound.* Acta. Neuropathol. Berl., 1983. **61**(2): p. 123-9.

[46]. McDonough, J., Jr., McLeod, C., Jr., and Nipwoda, M.T., *Direct microinjection of soman or VX into the amygdala produces repetitive limbic convulsions and neuropathology.* Brain Res., 1987. **435**(1-2): p. 123-37.

[47]. Wall, H.G., McLeod, C.G., Hutchison, L.S., and Shutz, M., *Brain lesions in rats surviving soman-induced convulsions: Light and electron microscopy.* Proc. USA Med. Def. Biosci. Rev., 1985: p. 457-473.

[48]. Baille-Le Crom, V., Collombet, J.M., Burckhart, M.F., Foquin, A., Pernot-Marino, I., Rondouin, G., and Lallement, G., *Time course and regional expression of C-FOS and HSP70 in hippocampus and piriform cortex following soman-induced seizures.* J. Neurosci. Res., 1996. **45**(5): p. 513-24.

[49]. Zimmer, L.A., Ennis, M., el Etri, M., and Shipley, M.T., *Anatomical localization and time course of Fos expression following soman-induced seizures.* J. Comp. Neurol., 1997. **378**(4): p. 468-81.

[50]. Zimmer, L.A., Ennis, M., Wiley, R.G., and Shipley, M.T., *Nerve gas-induced seizures: Role of acetylcholine in the rapid induction of Fos and glial fibrillary acidic protein in piriform cortex.* J. Neurosci., 1998. **18**(10): p. 3897-3908.

[51]. McDonough, J.H., Clark, T.R., Slone, T.W., Zoeffel, D., Brown, K., Kim, S., and Smith, C.D., *Neural lesions in the rat and their relationship to EEG delta activity following seizures induced by the nerve agent soman.* Neurotoxicology, 1998. **19**(3): p. 381-391.

[52]. Lallement, G., Pernot-Marino, I., Baubichon, D., Burckhart, M.F., Carpentier, P., and Blanchet, G., *Modulation of soman-induced neuropathology with an anticonvulsant regimen.* Neuroreport, 1994. **5**(17): p. 2265-8.

[53]. Carnevalli, L.S., Pereira, C.M., Jaqueta, C.B., Alves, V.S., Paiva, V.N., Vattem, K.M., Wek, R.C., Mello, L.E., and Castilho, B.A., *Phosphorylation of the alpha subunit of translation initiation factor-2 by PKR mediates protein synthesis inhibition in the mouse brain during status epilepticus.* Biochem. J., 2006. **397**(1): p. 187-94.

[54]. Wasterlain, C., *Inhibition of cerebral protein synthesis by epileptic seizures without motor manifestations.* Neurology, 1974. **24**: p. 175-180.

[55]. Lee, J.C., Laydon, J.T., McDonnell, P.C., Gallagher, T.F., Kumar, S., Green, D., McNulty, D., Blumenthal, M.J., Keys, J.R., Land vatter, S.W., Strickler, J.E., McLaughlin, M.M., Siemens, I.R., Fisher, S.M., Livi, G.P., White, J.R., Adams, J.L., and Young, P.R., *A protein kinase involved in the regulation of inflammatory cytokine biosynthesis.* Nature, 1994. **372**(29 Dec): p. 739 - 746.

[56]. Figiel, I. and Dzwonek, K., *TNFalpha and TNF receptor 1 expression in the mixed neuronal-glial cultures of hippocampal dentate gyrus exposed to glutamate or trimethyltin.* Brain Res., 2007. **1131**: p. 17-28.

[57]. Carballo, E., Lai, W.S., and Blackshear, P.J., *Feedback Inhibition of Macrophage Tumor Necrosis Factor-alpha Production by Tristetraprolin.* Science, 1998. **281**(5379): p. 1001-1005.

[58]. Sairanen, T.R., Lindsberg, P.J., Brenner, M., Carpen, O., and Siren, A.-L., *Differential cellular expression of tumor necrosis factor-[alpha] and Type I tumor necrosis factor receptor after transient global forebrain ischemia.* J. Neurol. Sci., 2001. **186**(1-2): p. 87-99.

[59]. Wong, D., Prameya, R., and Dorovini-Zis, K., *Adhesion and migration of polymorphonuclear leukocytes across human brain microvessel endothelial cells are differentially regulated by endothelial cell adhesion molecules and modulate monolayer permeability.* J. Neuroimmunol., 2007. **184**(1-2): p. 136-148.

[60]. Rebenko-Moll, N.M., Liu, L., Cardona, A., and Ransohoff, R.M., *Chemokines, mononuclear cells and the nervous system: heaven (or hell) is in the details.* Curr. Opin. Immunol., 2006. **18**(6): p. 683-689.

[61]. Turowski, P., Adamson, P., and Greenwood, J., *Pharmacological targeting of ICAM-1 signaling in brain endothelial cells: potential for treating neuroinflammation.* Cell. Mol. Neurobiol., 2005. **25**(1): p. 153-170.

[62]. Wu, J., Yang, S., Luo, H., Zeng, L., Ye, L., and Lu, Y., *Quantitative evaluation of monocyte transmigration into the brain following chemical opening of the blood-brain barrier in mice.* Brain Res., 2006. **1098**(1): p. 79-85.

[63]. Grilli, M., Barbieri, I., Basudev, H., Brusa, R., Casati, C., Lozza, G., and Ongini, E., *Interleukin-10 modulates neuronal threshold of vulnerability to ischaemic damage.* Eur. J. Neurosci., 2000. **12**(7): p. 2265-2272.

[64]. Bethea, J.R., Nagashima, H., Acosta, M.C., Briceno, C., Gomez, F., Marcillo, A.E., Loor, K., Green, J., and Dietrich, W.D., *Systemically administered interleukin-10 reduces tumor necrosis factor-alpha production and significantly improves functional recovery following traumatic spinal cord injury in rats.* J. Neurotrauma, 1999. **16**(10): p. 851-863.

[65]. Sawada, M., Suzumura, A., Hosoya, H., Marunouchi, T., and Nagatsu, T., *Interleukin-10 inhibits both production of cytokines and expression of cytokine receptors in microglia.* J Neurochem., 1999. **72**(4): p. 1466-71.

[66]. Williams, K., Dooley, N., Ulvestatd, E., Becher, B., and Antel, J.P., *IL-10 production by adult human derived microglial cells.* Neurochem. Int., 1996. **29**(1): p. 55-64.

[67]. Mizuno, T., Sawada, M., Marunouchi, T., and Suzumura, A., *Production of Interleukin-10 by Mouse Glial Cells in Culture.* Biochem. Bioph. Res. Co., 1994. **205**(3): p. 1907-1915.

[68]. Vila, N., Castillo, J., Davalos, A., Esteve, A., Planas, A.M., and Chamorro, A., *Levels of Anti-Inflammatory Cytokines and Neurological Worsening in Acute Ischemic Stroke.* Stroke, 2003. **34**(3): p. 671-675.

[69]. Tarkowski, E., Rosengren, L., Blomstrand, C., Wikkelso, C., Jensen, C., Ekholm, S., and Tarkowski, A., *Intrathecal release of pro- and anti-inflammatory cytokines during stroke.* Clin. Exp. Immunol., 1997. **110**(03): p. 492-499.

[70]. Hirayama, M. and Kuriyama, M., *MK-801 is cytotoxic to microglia in vitro and its cytotoxicity is attenuated by glutamate, other excitotoxic agents and atropine. Possible presence of glutamate receptor and muscarinic receptor on microglia.* Brain Res., 2001. **897**(1-2): p. 204-6.

[71]. Shimaoka, M., Lida, T., Ohara, A., Taenaka, N., Mashimo, T., Honda, T., and Yoshiya, I., *Ketamine inhibits nitric oxide production in mouse-activated macrophage- like cells.* Br. J. Anaesth., 1996. **77**(2): p. 238-242.

[72]. Sakai, T., Ichiyama, T., Whitten, C.W., Giesecke, A.H., and Lipton, J.M., *Ketamine suppresses endotoxin-induced NF-kappaB expression.* Can. J. Anaesth., 2000. **47**(10): p. 1019-1024.

[73]. Ghosh, S., May, M.J., and Kopp, E.B., *NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses.* Annu. Rev. Immunol., 1998. **16**: p. 225-260.

[74]. Won, S.J., Ko, H.W., Kim, E.Y., Park, E.C., Huh, K., Jung, N.P., Choi, I., Oh, Y.K., Shin, H.C., and Gwag, B.J., *Nuclear factor kappa B-mediated kainate neurotoxicity in the rat and hamster hippocampus.* Neuroscience, 1999. **94**(1): p. 83-91.

[75]. Hamilton, T.A., Ohmori, Y., and Tebo, J., *Regulation of chemokine expression by antiinflammatory cytokines.* Immunol. Res., 2002. **25**(3): p. 229-245.

[76]. Thomas, D.M. and Kuhn, D.M., *MK-801 and dextromethorphan block microglial activation and protect against methamphetamine-induced neurotoxicity.* Brain Res., 2005(1050): p. 190-198.

[77]. Kohrs, R. and Durieux, M.E., *Ketamine: teaching an old drug new tricks.* Anesth. Analg., 1998. **87**(5): p. 1186-1193.

[78]. Feinstein, D.L., Heneka, M.T., Gavrilyuk, V., DelloRusso, C., Weinberg, G., and Galea, E., *Noradrenergic regulation of inflammatory gene expression in brain.* Neurochem. Int., 2002. **41**(5): p. 357-365.

This work is supported by the French Ministry of Defence / DGA grant 03co011-05 to F. Dorandeu. The authors also wish to thank Dr Antonia Alonso and Ms Lucie Vachez-Collomb for their technical assistance in the protein assays.