RESEARCH ARTICLE

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Dose-effect study of the reversal by antimuscarinic agents of methomyl-induced respiratory toxicity

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ABSTRACT

Respiratory failure is the primary cause of death in poisonings involving carbamate insecticides. However, the mechanisms of respiratory toxicity induced by carbamates remain unclear. The aims of this study were i) to describe the respiratory effects of methomyl, ii) to assess the peripheral or central origin of those effects, and iii) to study the dose-effect relationship of atropine.

A dose of methomyl corresponding to 50% of the median lethal dose was given intraperitoneally to rats. Ventilation at rest was assessed using whole body plethysmography and core body temperature using infra-red telemetry. The central or peripheral origin was assessed comparing the effect of equipotent doses of atropine and methylatropine. The effects of dose of atropine ranging from 1 to 10 mg/kg were assessed. Total cholinesterase activities were determined using a radiometric method.

From 5 to 150 minutes post-injection, methomyl induced significant clinical symptoms, including significant decreases in respiratory frequency, which resulted from a significant increase in expiratory time. Methomyl induced a significant inhibition of brain total cholinesterase activities; meanwhile, atropine, but not methylatropine, completely reversed methomyl-induced respiratory toxicity. The dose-effect study showed that the efficient dose of atropine resulting in lowest adverse effects was 3 mg/kg while greater doses were efficient but induced significant adverse effects.

Decrease in brain cholinesterase activities accompanied by a positive effect of atropine, but not methylatropine, suggests a central origin of methomyl-induced respiratory toxicity. The 3 mg/kg single atropine dose yielded the best compromise between antidotal activity and intrinsic effects.

Introduction

Organophosphates and carbamates are widely used throughout the world as agricultural pesticides. Suicidal or inadvertent poisoning is a major health concern in the world as evidenced by the large annual incidence of acute life-threatening pesticide poisonings. Indeed, pesticide poisoning exacts a high mortality: mortality toll, with up to 200,000 deaths annually (1). Currently, organophosphate and carbamate pesticides remains the leading cause of toxic deaths in a number of countries, including North-Africa and Egypt (2) in addition to some Asian countries (3). Compared to organophosphates, carbamate pesticides are of more recent origin and constitute another important group of pesticides. Thousands of carbamates were synthesized. However, only a few dozen were of practical utility (4). As is the case for organophosphates, the potential to use carbamates by terrorists as a weapon of mass casualties might be considered as a major risk. In the Israeli

recommendations for treatment in mass casualty events involving carbamates, methomyl was classified as highly toxic (5). Indeed, among carbamates used as pesticides, methomyl is frequently involved in acute poisonings (1, 6-9) and indirectly, as a metabolite of thiodicarb(10).

Respiratory function is considered as the primary target in OP and carbamate poisoning and respiratory failure is the primary cause of death (11, 12). However, the knowledge of the mechanisms of carbamate-induced respiratory toxicity is still limited (11). The treatment of carbamate poisoning includes the combination of decontamination, supportive treatment, and the administration of atropine (1, 13). In human poisonings involving carbamate pesticides, the efficacy of atropine at high doses has been clearly demonstrated, however, using such doses can induce adverse effects (1).

In previous studies, we developed an experimental model to study the respiratory toxicity induced by diethylparaoxon in awaken rats (14) and a wide range of organophosphates of clinical interest, including dimethoate, fenthion, chlorpyrifos, and dichlorvos as these pesticides were frequently involved in acute poisonings in the Indian continent (3). The ventilatory effects induced by toxic but non-lethal doses diethylparaoxon included a decrease in respiratory rate and an increase in expiratory time that were completely reversed by a high dose of atropine but not by methylatropine at any tested doses (14, 15). Methylatropine was shown to produce peripheral anticholinergic effects comparable to those of atropine, without producing central anticholinergic effects (16). Rosnan*et al.* outlined that carbamate insecticides, including methomyl, generally do not cross the blood-brain barrier as organophosphates. Therefore, brain effects occur less frequently with lower severity than with organophosphates (5). We hypothesized that in the context of carbamate poisonings an anticholinesterase agent acting peripherically, including methylatropine, should reverse methomyl toxicity while preventing adverse effects related to centrally acting agents including atropine. Indeed, Eddleston*et al* pointed out there is a great variety of dosage regimens for atropine administration in poisonings involving anticholinesterase agents. This variability suggests both unsolved questions regarding atropine efficiency as well as safety (as regards the likelihood of occurrence of an atropine overdose following the time-course of pesticide poisonings) (17). Atropine overdose may of itself result in a life-threatening poisoning (17).

Therefore, the aims of this study were to determine the pattern of respiratory toxicity induced by a toxic, non-lethal dose of methomyl in the rat. Thereafter, the efficacy of atropine and methylatropine was compared with the aim of determining the central or peripheral origin of methomyl toxicity. Finally, as methylatropine failed to reverse methomyl-induced respiratory toxicity, we attempted to determine the lowest single dose of atropine able to reverse methomyl-induced respiratory toxicity, without inducing intrinsic effects.

Methods

1.1 Animals employed were male Sprague-Dawley rats (SociétéJanvier, Le Genest-St Isle, France) weighing between 250 and 350 g (age 8-12 weeks) at the time of experimentation. This study was approved by the Regional Committee for Animal Experimentation of University Paris Descartes (registration number P2.FB.050.08).

1.2 Median Lethal Dose (MLD) of methomyl. Administered by the intraperitoneal route was determined using the up-and-down method as proposed by Dixon (14, 18). Animals were examined repeatedly during the first 4-h period after injection, then daily for 7 days, for evidence of toxicant-related side effects or other illness.

1.3 Clinical examination was performed according to the method reported by De Candole*et al.* (12).

1.4 Whole body plethysmography. Ventilatory parameters, as frequency (f), inspiratory time (T_I), expiratory time (T_E), total time (T_{TOT}), tidal volume (V_T) and, minute ventilation (V_E), were recorded and calculated in a whole-body plethysmography device by the barometric method described and validated in the rat by Bartlett and Tenney(19) with minor modifications, as previously reported (14, 15). Central temperature of the rats was measured using infra-red telemetry, as previously described (15).

1.4.1 Effects of methomyl on respiration at rest in the rat: Ventilation at rest was studied in two groups of 6 animals. The control group received intraperitoneal isotonic saline solution (0.4 ml) instead of methomyl. The methomyl group received

methomyl 2.3 mg/kg (50% of the LD_{50}, equivalent to 0.3 to 0.5 ml). The first plethysmography measurement was performed after a period of accommodation of 30 minutes. Measurements were made three times to obtain baseline values. The animal was then gently removed from the chamber for the intraperitoneal injection and replaced in the chamber for another session of respiratory recording. Ventilation was recorded every 5 minutes during 30 min, every 10 min from 30 to 60 min and every 15 min from 60 to 180 min, each record lasting about 60 sec.

1.4.2 Reversal of methomyl-induced respiratory effects by equimolar dose of atropine or methylatropine: Ventilation at rest was studied in 5 groups of 6 animals. The control group received the solvent of methomyl (isotonic saline solution, 0.4 ml) followed 10 minutes after by the solvent of atropine/methylatropine (isotonic saline, 0.4 ml). The methomyl group received methomyl (2.3 mg/kg) followed 10 minutes after by the solvent of atropine/methylatropine (isotonic saline, 0.4 ml). For all groups, methomyl was administered at the same dose (2.3 mg/kg) as that used in the study 2). Atropine (10 mg/kg) or methylatropine (5.42 mg/kg) was administered intramuscularly 10 minutes post-methomyl injection. The plethysmography measurements were performed as previously described.

1.4.3 Dose-effect relationship of a single dose of atropine in the model of methomyl poisoning

A single dose of atropine, 1, 3, 5, 7, or 10 mg/kg, was administered intramuscularly 10 minutes post-methomyl injection. Each group included 5 animals.

1.5 Measurement of total cholinesterase (TChE)activities were performed in of whole blood and exsanguinated tissues using the radiometric method, as previously described (20). Total cholinesterase activities were expressed as the percent of control activity. Total cholinesterase activities were determined 30 min after methomyl administration (n=5), when methomyl-induced ventilatory effects reached their plateau.

1.6 Statistical analysis. All results are expressed as mean ± SEM. Graphs and statistical analysis were performed using Prism version 5.0 GraphPad Software (San Diego, CA). As requested, statistical analysis was performed using a two-way analysis of variance for repeated measurements. A p-value of less than 0.05 was considered significant. For parameters with a significant treatment*time interaction, the analysis was followed by multiple Bonferroni comparison tests. For the study of the effects of ascending doses of atropine, experimental areas under the curve (EAUCs) were calculated from 10 to 180 minutes ($EAUCs_{10-180}$) using the trapezoidal method (21) after methomyl, antidote, and solvent administrations. AUCs were compared using a one-way analysis of variance followed by multiple Bonferroni comparison tests.

Results

2.1 Median Lethal Dose

The median lethal dose (MLD) by the intraperitoneal route was 4.60 mg/kg.

2.2 Effects of methomyl on respiration at rest in rat

Regarding baseline values,there were no significant differences of the temperature, the f, T_{TOT}, T_E, T_I, V_T, and V_E comparing the control and methomyl groups.

In comparison with the control group, the rats poisoned with methomyl exhibited, within 5 minutes of injection, hypersalivation, diaphoresis, urinary and fecal incontinence, tremors and fasciculations. Clinical signs spontaneously resolved at approximately hundred 50 minutes.

During the study period, the core temperature in the control group remained normal and stable, with a mean value of 37.6 ± 0.1°C (Fig. 1). In the methomyl group, there was a decrease in the core temperature which became significantly lower than the value in the control group at 10 min after methomyl injection (36.7 ± 0.1 °C *versus* 37.8 ± 0.1 °C in the control group, $p< 0.05$). The minimal value was

observed at 90 min (35.2 ± 0.2 °C in the methomyl group *versus* 37.5 ± 0.1 °C in the control group, p< 0.01). The temperatures remained significantly lower than the values in the control group up to the completion of the study. At the end of the study, the mean temperatures in the methomyl and control groups were 36.3 ± 0.2°C and 37.4 ± 0.4 °C (p< 0.05), respectively (Fig. 1). No animal died during the study.

Fig 1

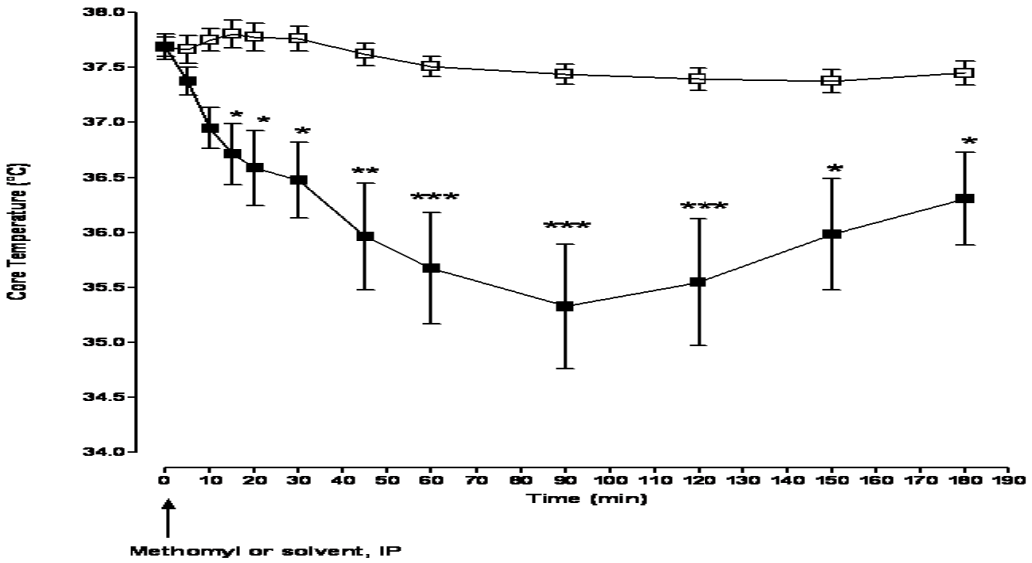

*Fig. 1: Time-course of core temperature in control rats (open squares) and methomyl-poisoned rats (dark squares).Statistical significance: * p< 0.05, ** p< 0.01,*** p< 0.001 methomyl versus control group.*

Plethysmography study showedat 5 min after methomyl injection, in comparison with the control group, total respiratory time (T_{TOT}), expiratory time (T_E), and, Tidal volume (V_T)values were significantly increased (p< 0.001) in the methomyl group: 0.71 ±0.03 *versus* 0.55 ± 0.01 s, 0.51 ± 0.02 *versus* 0.37 ± 0.01 s, and 3182 ± 204 *versus* 1978 ± 31 µl, respectively (Fig. 2). Conversely, frequency was significantly lower (p< 0.001) in the methomyl*versus* control groups: 87 ± 4 *versus* 111 ± 3 cycles/min, respectively. Ventilatory parameters remained significantly different from control values until 60 (V_T), 90 (T_E), and 120 minutes (f, T_{TOT}) after methomyl injection. At the end of the experiment, at 150 minutes, ventilatory parameters had spontaneously returned to control values. There were no significant differences of the inspiratory time (T_I) and

minutevolum
e (V_E) in the two groups at any time (Fig. 2).

Fig 2

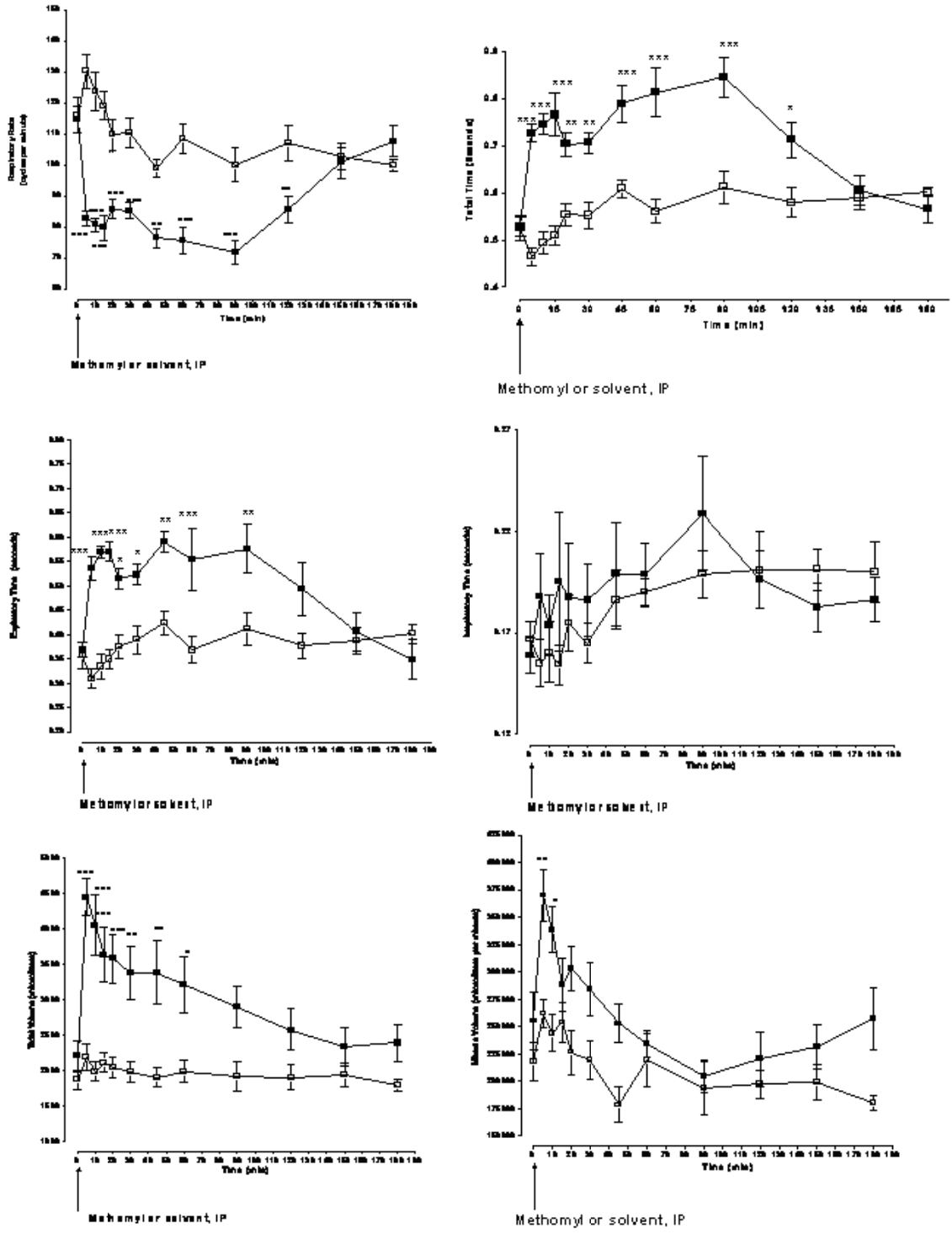

*Fig. 2: Time-course of respiratory rate (f), total time (T_{TOT}), expiratory time (T_E), inspiratory time (T_I), Tidal volume (V_T), and minute volume (V_E) in control rats (open squares) and methomyl-poisoned rats (dark squares). Statistical significance: * p< 0.05, ** p< 0.01, *** p< 0.001 methomylversus control group.*

Thirty minutes after administration of methomyl, TChEresidual activities in whole blood, brainstem, thigh muscle, and frontal brain were 28 +/- 3 %, 26+/- 2 %, 26+/- 2 %, and 32 +/- 3% of baseline, respectively (Fig. 3).

Fig 3

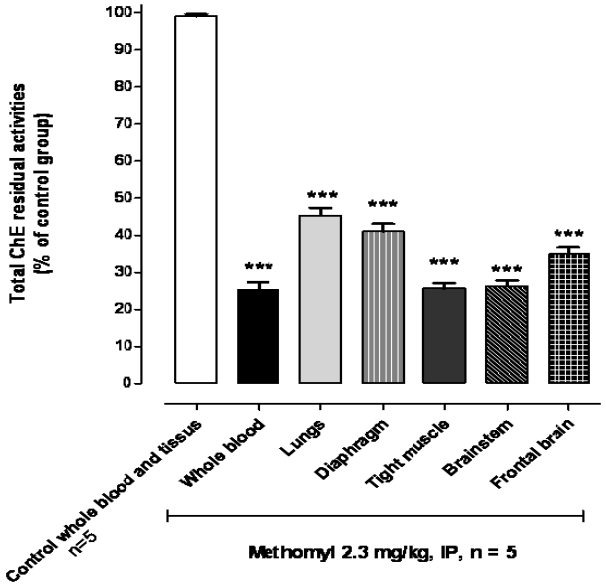

*Fig. 3: Total cholinesterase residual activities in whole blood and tissues in methomyl-poisoned rats. Statistical significance: *** p< 0.001 methomylversus control group.*

2.3 Reversal of methomyl-induced respiratory effects by of atropine or methylatropine:

Regarding baseline values,there were no significant differences of the temperature, the f, T_{TOT}, T_E, T_I, V_T, and V_E comparing the control and all treated groups.

Clinical f indings,including hypersalivation, urination, fecal incontinence, tremor, and fasciculations induced by methomyl, were completely corrected between 80 (10 mg/kg) and 120 min (3 mg/kg) after atropine administration. Only hypersalivation, urinary, and fecal incontinence were corrected by the lowest dose of 1 mg/kg. At the opposite, methylatropine did not modify any signs and symptoms in comparison with the methomyl group.

During the study period, methomyl induced a drop in core temperature which was not corrected by methylatropine. Only, the 10 mg/kg dose of atropine significantly corrected drop in core temperature from 60 minutes post-methomyl injection to the end of the experiment (Fig. 4).

Fig 4

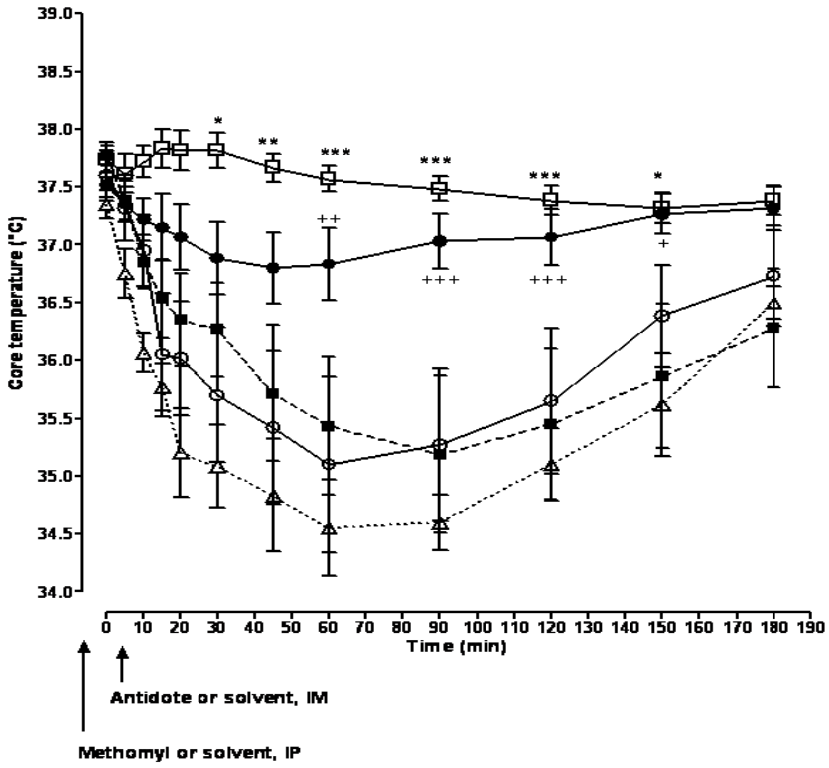

*Fig.4: Time-course of core temperature in control rats (open squares), methomyl-poisoned rats (dark squares), in 10 mg/kg atropine-treated rats (dark circles), in 5.42 mg/kg methylatropine-treated rats (open circles) and in 50 mg/kg pralidoxime-treated rats (open triangles). Statistical significance: * p< 0.05, ** p< 0.01, *** p< 0.001 control versusmethomyl group; + p< 0.05, ++ p< 0.01, +++ p<0.001 atropine versusmethomyl group*

Plethysmography study showed thatbefore antidote administration, the values of f, T_{TOT}, T_E, T_I, V_E, and V_T in the atropine, and methylatropine groups were not significantly different from those in the methomyl group. After administration of atropine, there were progressive improvements in the values of the f, T_{TOT}, and T_E so that they became significantly different from the values in the methomyl group at 20 min after methomyl injection ($p< 0.01$) At this time, no ventilatory parameters were significantly different from controls. From 30 to 180 minutes after injection of methomyl, values of the respiratory parameters in the atropine group were greater (f) or lower (T_{TOT}, T_E,) than those in the control group values. After administration of methylatropine (5.42 mg/kg) there were no significant corrections of methomyl modified respiratory parameters, at any time (Fig.5).

Fig 5

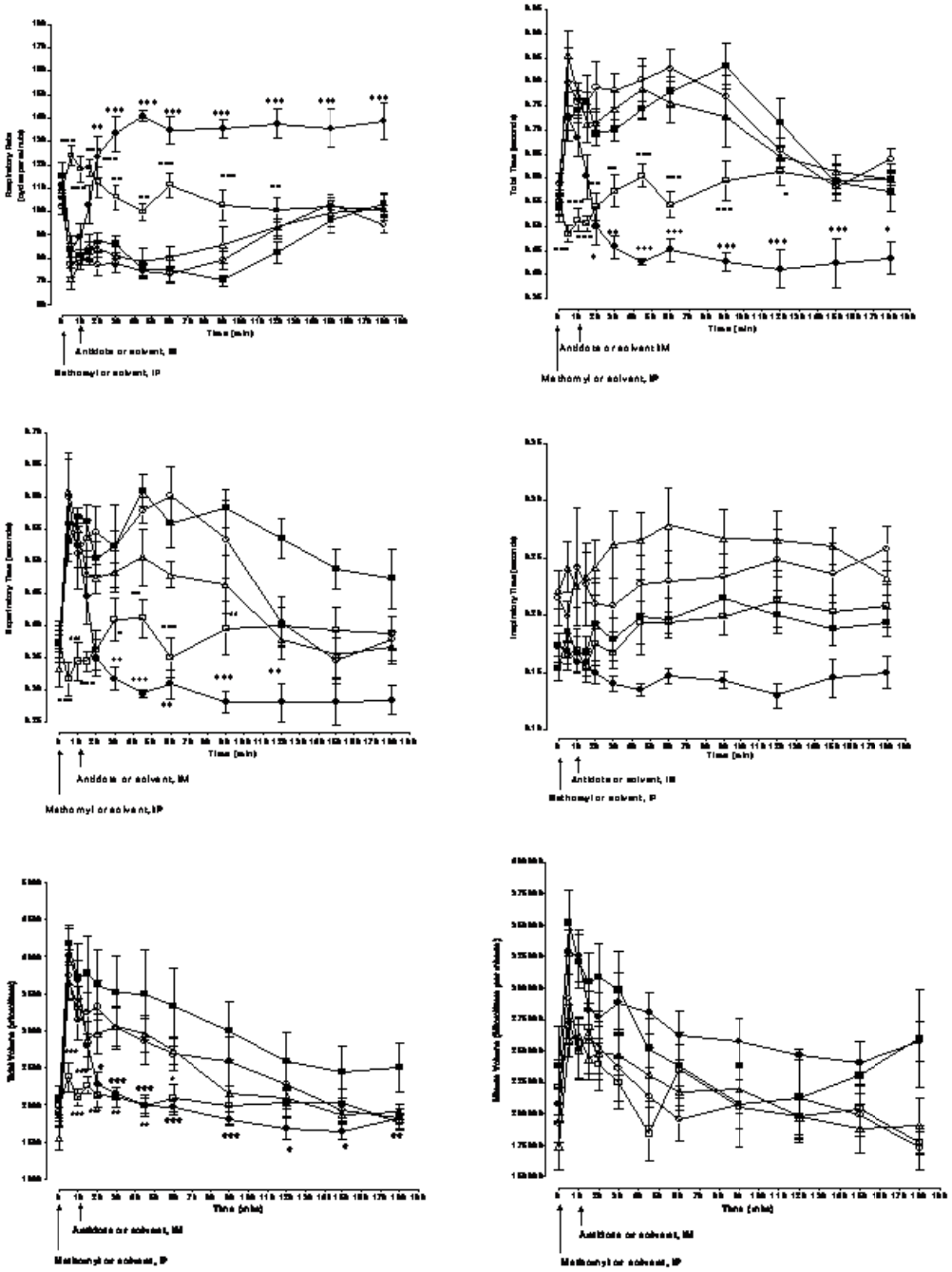

*Fig.5: Time-course of respiratory rate (f), total time (T_{TOT}), expiratory time (T_E), inspiratory time (T_I), Tidal volume (V_T), and minute volume (V_E) in control rats (open squares) and methomyl-poisoned rats (dark squares), in 10 mg/kg atropine-treated rats (dark circles), in 5.42 mg/kg methylatropine-treated rats (open circles) and in 50 mg/kg pralidoxime-treated rats (open triangles). Statistical significance: * p< 0.05, ** p< 0.01, *** p< 0.001 control versusmethomyl group; + p< 0.05, ++ p< 0.01, +++ p<0.001 atropine versusmethomyl group*

2.4 Dose-effect relationship of a single dose of atropine in the model of methomyl poisoning

The dose-effect study showed in the group receiving the lowest atropine dose (1 mg/kg), that the mean experimental area under curves from 10 to 180 minutes ($EAUCs_{10\text{-}180}$) was not significantly different from that in the methomyl group for f, T_{TOT}, T_E, and V_T (Fig. 6). In the 3 mg/kg dose group, the mean $EAUCs_{10\text{-}180}$ value was significantly different from that in the methomyl group but not from those in the control group for respiratory as parameters f, T_{TOT}, T_E, and V_T . For atropine doses ranging from 5 to 10 mg/kg, the mean $EAUCs_{10\text{-}180}$ values were significantly different in comparison with the methomyl and the control groups. For the doses greater than 3mg/kg, the $EAUCs_{10\text{-}180}$ demonstrated a greater decrease than those in the control values for T_{TOT}, T_E, and conversely, an increase in f. Neither methomyl poisoning nor atropine administration, whatever the dose, induced any significant effects on the mean $EAUCs_{10\text{-}180}$ values for inspiratory time and minute volume (Fig. 6).

Fig 6

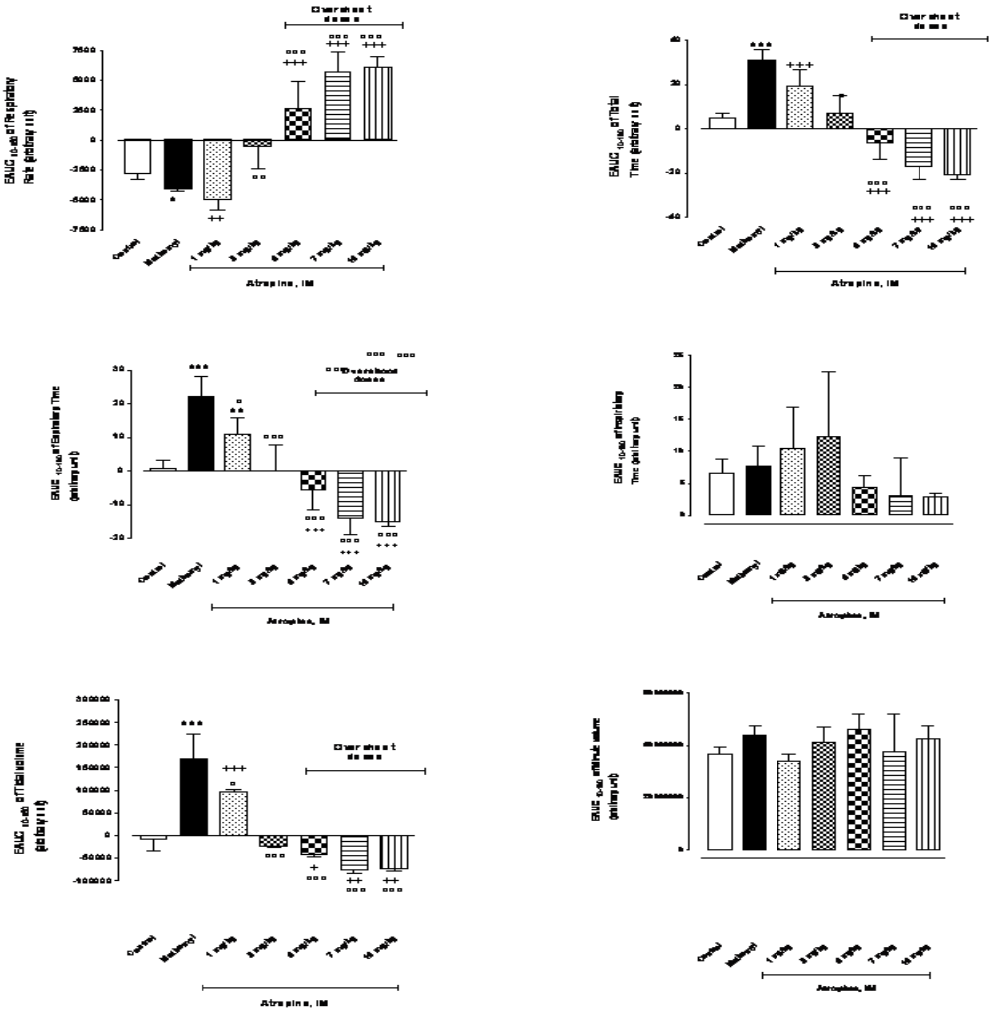

Fig. 6: Areas under the curves of the effects from 10 to 180 minutes ($EAUCs_{10\text{-}180}$) for dose-response relationship of ventilatory parameters in control rats (white bar), methomyl-poisoned rats (black bar), atropine 1 mg/kg-treated rats (diamond), atropine 3 mg/kg-treated rats (diagonal stripes), atropine 5 mg/kg-treated rats (black and white square), and atropine 7 mg/kg-treated rats (horizontal stripes), atropine 10 mg/kg-treated rats (vertical stripes).

*Statistical significance: * p< 0.05, ** p< 0.01, *** p< 0.001 methomyl or atropine doses versus control group; + p< 0.05, ++ p< 0.01, +++ p<0.001 control or atropine doses versusmethomyl group*

Discussion

While respiratory failure is considered the primary cause of death in acute organophosphate and carbamate poisonings (1, 22,23). The respiratory effects and the mechanisms of toxicity of the inhibitors of cholinesterases still remain poorly documented (24-26). Consequently, we performed a series of studies designed to clarify the respiratory effects induced by a fixed toxic but non-lethal dose using for each studied compound an equipotent dose equal to half the MLD determined in our laboratory using male Sprague-Dawley rats. The studied compounds included ethyl-oxon, including diethylparaoxon, methyl-oxon, including dimethylparaoxon, and ethyl-thion, including fenthion, dimethoate, and chlorpyrifos(27) . At this dose, all the studied anticholinesterasic agents induced the same toxicological profile, including a decrease in respiratory rate and an increase in the tidal volume resulting in an unchanged expired minute ventilation. The decrease in respiratory rate resulted from an increase in the expiratory time meanwhile the inspiratory time remained unchanged. The magnitude of changes in ventilation at rest were within the same range for all compounds at this equipotent dose equal to half the MLD. In contrast, the time-course of effects, were quite different with rapid-acting compounds including dichlorvos and dimethylparaoxon for which the onset of maximal effects occurred within 10 minutes post-subcutaneous injection, intermediate compounds, including diethyl-paraoxon for which the onset of maximal effects occurred within 30 minutes post-subcutaneous injection, and delayed-acting compounds including dimethoate, chlorpyrifos, and fenthion for which the onset of maximal effects occurred within 90 minutes post-subcutaneous injection. As expected, thion-derivatives were delayed acting compounds. Methomyl acted similarly as short-acting compounds. Regarding the late phase of poisoning, dichlorvos induced the shortest duration of alteration of ventilation at rest, spontaneously lasting less than 30 minutes in comparison with the others in which the duration of effects lasted hours. In comparison with the durations of respiratory effects induced by organophosphates, the duration of the effect induced by methomyl lasted within 150 min, i.e. longer than dichlorvos but far shorter than the other organophosphates.

The mechanisms of respiratory failure induced by pesticides remain unclear. Several hypotheses have suggested both a peripheral origin, including diaphragm and respiratory musculature paralysis associated with increased secretions by the airways and a central origin by action on the control of breathing (6, 9). Limited knowledge in the mechanisms of toxicity is further supported by the conflicting results regarding the efficiency of pralidoxime in organophosphate poisoning (3, 28,29). The need for improving knowledge in the mechanism of toxicity and treatment is further supported by the recent confirmed events of the use of nerve agents as chemical weapons, resulting in hundreds of deaths. Noteworthy, regarding the estimated toxic potency, methomyl is classified one among the most highly toxic carbamate insecticides in human which may be used as a surrogate of chemicals weapons (5).

The study of chemicals acting on the control of breathing is rather difficult, more especially as general anesthesia dramatically alter the effects of toxicants inducing own effects in addition to those induced by toxicant (30). To address this difficulty resulting from general anesthesia, in the present study whole body plethysmography was used in awake animals at rest (14, 31). To prevent any concern regarding the validity of our results, we considered it mandatory to assess the MLD in the rat species we used. The observed MLD of methomyl was 4.6 mg/kg. This value is close to the 6.3 mg/kgvalue previously determined using the same method and the same route of administration in female albino Wistar rats (32) meanwhile, we used male Sprague-Dawley rats.

Carbamate insecticides are generally reported as unable to cross the blood-brain barrier as easily as organophosphates do and thus, less frequently inducing central nervous signs (33). However, seizures were consistently reported in methomyl poisoning suggesting its ability to result in central effects (34). In the present study, the

MLDof methomyl induced significant muscarinic and nicotinic toxic signs, as well as a decrease in blood and tissue total cholinesterase activities. Indeed, we reported a significant inhibition of total cholinesterase activity in the brain (32 +/-3 % of baseline activity) and brainstem (26 +/- 2% of baseline activity) after methomyl poisoning. Similar results regarding methomyl-induced cholinesterase inhibition in the brain were reported in rodents after oral doses of 3 mg/kg (35) and 2.5 mg/kg of methomyl(36). These data support the assumption that methomyl does efficiently cross the blood-brain barrier, at least at the studied doses and in the rat. This hypothesis is further supported by the efficiency of atropine and the lack of efficiency of an equipotent dose of methylatropine. The inefficiency of methylatropine might be explained by the fact that this ammonium compound cannot cross the blood-brain barrier, as previously reported in diethylparaoxon-induced ventilatory effects (15).

The present study showed that methomyl-induced toxicity was comparable to that previously reported regarding the magnitude of signs and symptoms, hypothermia (27, 37, 38) and the degree of cholinesterase inhibition (39) after organophosphate poisoning in the rats. Indeed, after methomyl exposure, we observed a decrease in respiratory rate, an increase in the expiratory time, and in the tidal volume. Interestingly, this equipotent dose resulted in the magnitude of effects that were similar to those previously reported using equipotent doses of the various organophosphates (14, 15). However, the time-course of respiratory toxicity induced by methomyl in rats was close to that induced by rapid-acting organophosphate, including dichlorvos(27). Meanwhile the duration of methomyl-induced respiratory toxicity of about 150 min was longer than that induced by dichlorvos and far shorter than that induced by the other organophosphates. Our present and previous experimental results (14, 15, 27, 40) are consistent with human data showing similar respiratory patterns induced by organosphophorus compounds and carbamates but with different time-course of effects (22, 23). This difference in the time-course of events is supported by previous reports in humans poisoned with methomyl. Indeed, methomyl-induced toxicity was shown to have a shorter duration than those seen after organophosphate poisoning (5) due to a fast cholinesterase-inhibited spontaneous re-activation (1). One limit of this study results from the fact we did not determine the level of spontaneous reactivation with time. In rats, spontaneous reversion was reported to only be completed within 24 hours after methomyl (3 mg/kg) ingestion as reported by Padilla *et al*(35).

In contrast with organophosphate poisoning, atropine is considered as the single antidote in addition to supportive treatment in carbamate poisoning (1, 5,13). The efficiency and safety of pralidoxime in carbamate poisonings remains controversial (5, 28). In our rat model, a 10 mg/kg dose of atropine base resulted in a complete reversal of clinical effects, and a complete reversal of methomyl-induced respiratory effects within 10 minutes after atropine injection. In contrast, the same dose of atropine reversed the respiratory toxicity induced by an equipotent dose of diethylparaoxon completely only at 30 min post paraxoxon-injection (15). Regarding hypothermia which is an effect consistently reported in both organophosphate and carbamate poisonings, the present study showed a partial reversal of hypothermia with atropine which was similar to that observed in chlorpyrifos-poisoned rats treated with scopolamine (41).

High doses of atropine are recommended in the treatment of carbamate poisoning (5) with the risk of severe adverse effects, however (17). To determine the balance between efficiency and adverse effects of atropine, we studied the dose-response relationship. Owing to the duration of methomyl-induced respiratory toxicity, we assessed the therapeutic effects of a single dose of atropine. Only the 3 mg/kgdose resulted in the complete reversal of methomyl-induced toxicity without any overshoot effect as assessed using the $AUCs_{10\text{-}180}$ of effects. Indeed, greater doses than the 3 mg/kg dose of atropine resulted in $EAUCs_{10\text{-}180}$ values that were significantly lower than those in the control values for T_{TOT}, $T_{E.}$ Indeed, a greater of atropine resulted in a significant increase of f. This feature suggests an overshoot effect of atropine likely exhibiting intrinsic toxicity. The 3 mg/kg dose determined in this study is smaller than that reported in the literature. Indeed, doses of atropine ranging from 8 to 17.4 mg/kg were necessary to reverse carbamate toxicity in rodents (42-44). However, the previously

reported studies focused only on the efficiency of atropine while not taking into account for the adverse effects of atropine.

Our study suffers from a number of limitations. We did not use the colorimetric method which is considered as the method of reference (45). Indeed, the colorimetric method can be performed without restriction meanwhile the radiometric method uses radio-activity and must be restricted to authorized laboratory. However, the colorimetric method uses acetylthiocholine as the substrate instead of acetylcholine which is the physiological substrate. We and others used the radiometric method owing to its specificity for acetylcholine more especially as we measured different forms of cholinesterases existing in the blood and tissues of rats (20, 46). Indeed, the esterase activities are mainly carboxyesterase in blood, and butyrylcholinesterase in the other tissues except in the brain where acetylcholinesterase is the major detoxifying enzyme (47). In the present study, we did not test the efficiency of oximes. However, in carbamate intoxications, the use of atropine is considered as the main treatment, whereas the use of oxime therapy is still controversial (1, 5) .An increase in toxicity when pralidoxime was used to counteract toxicity of aldicarb in experimental studies (28). Oximes was reported to reinforce the inhibition of cholinesterase in humans (17, 48). However, the later assumption is supported by a limited number of experimental as well as human data. Therefore, further studies are needed to assess the efficiency of oximes in anticholinesterasiccarbamate poisonings. We tested only one dose of methomyl. However, in the present study, we used the same fixed dose allowing comparison of effects between organophosphate as well as carbamate insecticides.

Short-acting anticholinesterasic agents, including dichlorvos and methomyl, progressively replaced long-acting agents in a number of developing countries. In spite of this modification, anticholinesterasic agents remain a major class of toxicants involved in acute poisonings resulting in admission in intensive care units (49). Except for temperature, clinical signs and respiratory effects spontaneously resolved within 150 min post-injection. Reversal of clinical and respiratory signs can be explained by the rapid and spontaneous decarbymalation of esterase enzymes induced by carbamates(36). However, in our study, total cholinesterase activities were not measured at 150 min. Therefore, we cannot verify this hypothesis.

Further studies are needed before the present result can be extended to human poisonings.

In conclusion, the present study showed a 2.3 mg/kg dose of methomyl, corresponding to half the MLD measured in our laboratory, was used to assess the dose-effect relationship of atropine in the reversal of methomyl-induced respiratory toxicity. In contrast with diethylparaoxon-induced respiratory toxicity, methomyl-induced respiratory effects occurred sooner and spontaneously reversed within 150 minutes without any treatment. Atropine, but not methylatropine completely reversed methomyl-induced respiratory toxicity, supporting the hypothesis of central effects of methomyl. The central effect of methomyl is further supported by the decrease in all brain cholinesterase activities, including the brainstem. The dose-effect study showed that the efficient dose of atropine resulting in lowest adverse effects was 3 mg/kg while greater doses were efficient but induced significant intrinsic effects.

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