INTERACTION BETWEEN THIOPENTAL AND FENTANYL IN MICE IS DIFFERENT BETWEEN HYPNOTIC AND LETHAL DOSES

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SUMMARY

Much is known about the interaction of intravenous anesthetics and opioids at the therapeutic level, but less is known regarding their combined lethal effect, leaving some uncertainty regarding the window of safety for their clinical use. We set out to document the type of interaction between thiopental and fentanyl for both the hypnotic effect (loss of righting reflex) and lethal effect in mice. Hypnotic and lethal dose-response curves were constructed for thiopenthal alone and in combination with fentanyl (0.8 μg/kg, each based on five to seven subgroups of six to ten ICR mice. The dose of fentanyl was that needed to double the lag time to tail flick following a noxious stimulus (the equivalent of human analgesia). While fentanyl did not change the median effective hypnotic dose of thiopental (8.9 mg/kg [95% confidence interval {CI} 8.0-9.9 mg/kg] alone versus 7.8

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mg/kg [95% CI 6.7-8.7 mg/kg] in combination), it significantly reduced its median lethal dose from 71.8 mg/kg (95% CI 68.3-74.8 mg/kg) to 64.5 mg/kg (95% CI 63.7-65.2 mg/kg). Most remarkably, it increased the slope of the curve from 0.17 (95% CI 0.10-0.36) to 0.61 (95% CI 0.24-1.10), virtually eliminating the difference between the non-lethal and lethal ranges. We conclude that the type of interaction between thiopental and fentanyl is stronger for the lethal effect than for the hypnotic effect. This may become relevant to clinical situations in humans when higher doses of thiopental are used.

KEY WORDS

sodium thiopental, fentanyl, interaction, hypnotic effect

INTRODUCTION

Combinations of intravenous (i.v.) opioids are part and parcel of balanced anesthesia. Much is known about their interaction at the therapeutic level but less is known regarding their combined effect at the lethal level. In the current study we attempted to document the type of interaction between two widely used and frequently combined drugs, thiopental and fentanyl, for both hypnotic and lethal effects in non-ventilated mice. This documentation was expected to delineate the projected 'window of safety' for clinical use in humans.

MATERIALS AND METHODS

Experiments were performed in ICR mice weighing 20-30 g. Their treatment was in accord with the Guide for the Care and Use of Laboratory Animals, and the study was approved by the Animal Care and Use Committee of the Technion Faculty of Medicine. The mice were allowed to escape into a dark tube, where they were secured by an adhesive band through which the tail was passed. Thiopental and fentanyl were injected into the tail vein after vasodilating it under a 40-W lamp for 2 min. The injection volume was kept constant at 10 ml/kg (0.2-0.3 ml) to avoid hemodynamic differences between groups. A dose-response curve for the loss of righting reflex 15 sec after injection of thiopental was calculated from eight dose groups of eight

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mice each (6, 7, 8, 10, 11, 12, 13, and 15 mg/kg). Since some mice were noted to lose the righting reflex at these doses but to regain it earlier than 15 sec, we also constructed a curve for loss of this reflex at 8 sec, with doses of 6, 7, 8, 10, and 11 mg/kg. This was based on the assumption that regaining the righting reflex represented redistribution of thiopental. In all further combination studies, we kept this dual evaluation at 8 and 15 sec.

A dose-response curve of the analgesic effect of fentanyl was calculated from six dose groups of five mice each (0, 0.5, 0.75, 1.0, 1.25, and 1.5 μg/kg). The test examined the lag time between dipping the tail in a 55°C water bath and the tail-flick response. In a preliminary set of observations, we noted that some mice flicked their tail immediately upon sensing the water and not as a response to the excessive temperature. Thereafter, tails were briefly dipped into the water bath for preconditioning before the test itself.

Dose-response curve for the lethal effect of thiopental was based on five dose groups of eight mice each (50, 65, 70, 75, and 80 mg/kg). Since deaths of non-ventilated animals undergoing anesthesia may result from immediate cardiac arrest or prolonged respiratory depression, we carried out a special methodological evaluation before further testing. In preliminary experiments we observed that many mice that did not die immediately, i.e., within 0.5 to 5 min after injection of thiopental, went through a phase of profound bradycardia and bradypnea lasting up to 15 min. After this, they recovered and regained normal activity. This suggested that, most probably, immediate death occurred as a result of cardiac toxicity rather than of respiratory depression. Additionally, mice that died within the immediate period did not bleed from the venipuncture point, as opposed to mice that recovered, further supporting the possibility of cardiac standstill. In an attempt to verify the exact mechanism leading to death, we took an electrocardiographic recording after injection of a lethal dose. This revealed continuation of electrical complexes despite no palpable heartbeat through the chest wall. Thoracotomy within 5-10 sec after injection of a lethal dose revealed ventricular standstill, despite continuation of some atrial movement for about 1 minute. Cumulatively, these observations suggested electromechanical dissociation. Our final validation step used an echocardiographic approach. Mice were followed by an M-mode echocardiogram, using the 9-5 MHz probe (ATL, Bothell, WA). It was clearly observed that cardiac

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arrest occurred about 4 sec after injection of the lethal dose of thiopental, which was too early to be secondary to respiratory depression.

In a second set of experiments, the dose-response curves of thiopental in combination with 0.8 μg/kg fentanyl (the dose which doubled the lag time of tail flick) were calculated for both loss of righting reflex and lethal effect.

Probit and bootstrap analyses were used to calculate the median effective dose (ED_{50}) and median lethal dose (LD_{50}), to estimate the significance of the dose-response relationships, and to obtain the 95% confidence interval (CI) for the slope of the curves /1/. The probit curves of thiopental alone and in combination with fentanyl were then compared to estimate the difference between their respective slopes.

RESULTS

The calculated ED_{50} of thiopental for loss of righting reflex was 10.8 mg/kg (95% CI 10.0-11.8 mg/kg) and 8.9 mg/kg (95% CI 8.0-9.9) for 15 and 8 sec, respectively. Both curves showed a significantly good fit to a straight line ($r^2 > 0.8$; $p < 0.04$). The respective slopes were 0.44 (95% CI 0.31-0.87) and 0.55 (95% CI 0.33-1.30), which were not significantly different from each other (Fig. 1).

The results of the tail-flick lag-time test to evaluate the analgesic effect of fentanyl are presented in Figure 2. The regression line was statistically significant ($r^2 = 0.62$; $p < 0.001$). The calculated dose that doubled the tail-flick lag time was 0.8 μg/kg (95% CI 0.43-1.20).

ED_{50} of thiopental for the loss of righting reflex when combined with fentanyl 0.8 μg/kg was 10.0 mg/kg (95% CI 9.2-11.1 mg/kg) and 7.8 mg/kg (95% CI 6.7-8.7 mg/kg) at 15 and 8 sec, respectively. The slopes were 0.40 (95% CI 0.25-0.65) and 0.46 (95% CI 0.28-0.99) (Fig. 3). There was no significant difference between the ED_{50}s and slopes of the curves of thiopental alone and in combination with fentanyl.

The calculated LD_{50} of thiopental alone was 71.8 mg/kg (95% CI 68.3-74.9 mg/kg), compared with 64.5 mg/kg (95% CI 63.7-65.2 mg/kg) when combined with fentanyl 0.8 μg/kg, which was significantly different. The respective slopes were 0.17 (95% CI 0.10-0.36) and 0.61 (95% CI 0.24-1.10), which were significantly different

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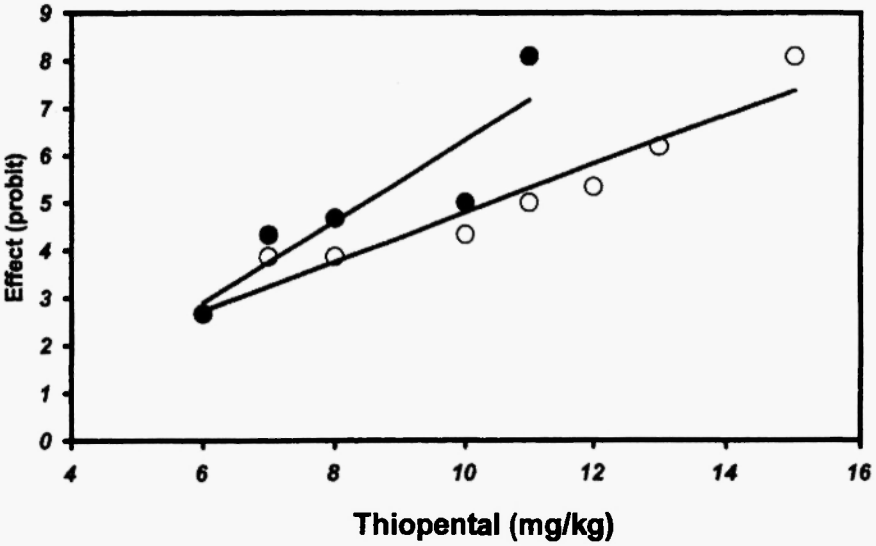

Fig. 1: Dose-response curves of i.v.-administered thiopental for the loss of righting reflex in mice after 8 sec (solid circles) and 15 sec (open circles). Each symbol represents a group of eight animals.

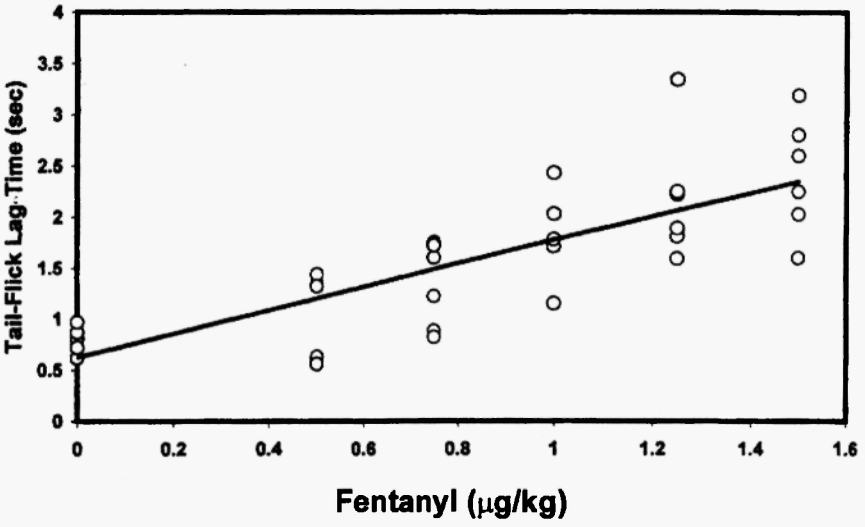

Fig. 2: Dose-response curves of i.v.-administered fentanyl for tail-flick lag time in mice. Each symbol represents a group of eight animals.

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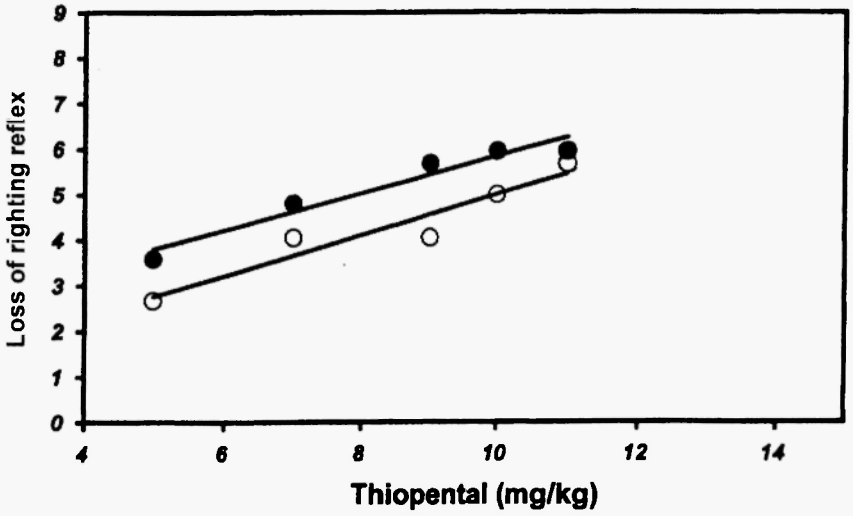

Fig. 3: Dose-response curves of i.v.-administered thiopental in combination with i.v. fentanyl 0.8 µg/kg after 8 s (solid circles) and 15 s (open circles) for the loss of righting reflex in mice. Each symbol represents a group of 10 animals.

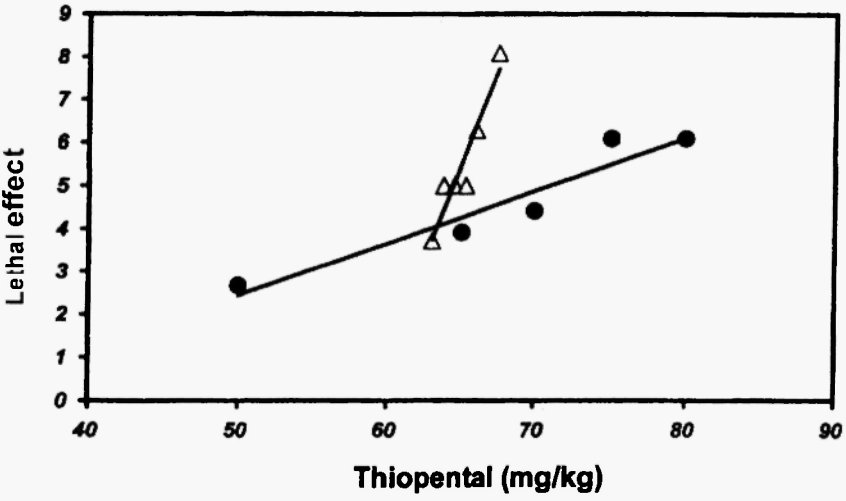

Fig. 4: Dose-response curves of i.v.-administered thiopental (closed circles) or in combination with i.v. fentanyl 0.8 µg/kg (open triangles) for the lethal effect in mice. Each symbol represents a group of 10 animals.

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(Fig. 4). SD of the slope for thiopental alone was 0.072 and for thiopental plus fentanyl 0.193.

DISCUSSION

Sedative-hypnotics and opioids are combined for premedication, induction, and maintenance of almost every anesthesia. Automated i.v. infusion devices that can support precise titration have popularized the use of these combinations in maintenance of anesthesia. Interaction between these two drugs, which share a common endpoint, can accumulate to be antagonistic, additive, or synergistic towards that point. It is generally assumed that whereas the therapeutic effect adds up to a known degree, the toxicity is proportional only to the fraction of each drug involved. This has been illustratively described as widening of the 'therapeutic window' between the effective dose and toxicity. The literature is rich in reports on studies that addressed the interaction at the therapeutic level, i.e., hypnosis and/or analgesia, in animals /2-4/ as well as in humans /5/. Although certain drugs have been noted to combine in reducing the shared cardiovascular depressive side effects, as in the case of fentanyl and thiopental /6-10/, too little has been done to evaluate in a systematic way the type of interaction when death (reflecting the ultimate toxicity) serves as the endpoint. To accomplish the latter, one should adopt a relevant animal model for delineation of the therapeutic window. In rodents, loss of righting reflex is taken as an equivalent of hypnosis /3/ and tail-flick latency as an estimator of analgesia /2/. Thus, if a combination of two drugs interacts towards hypnosis in a certain pattern in both an animal model and humans, it can reasonably be assumed that the pattern of interaction at the toxic level is also relevant to humans.

In the present study we found minor, non-significant changes in the ED_{50} of thiopental for the loss of righting reflex in mice following addition of an analgesic dose of fentanyl. The similarity of the slopes of these curves suggests that the pharmacodynamics of thiopental's hypnotic effect is not changed by the addition of fentanyl. In a remarkable contrast, the lethal dose-response curve of thiopental was considerably shifted to the left by the addition of the same dose of fentanyl (LD_{50} 71.8 and 64.5 mg/kg, respectively). Moreover, the slope of the curve was significantly increased. This may indicate that at the lethal level the pharmacodynamic interaction between the two

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drugs is materially different from that at the hypnotic level. If this is expected to hold true for humans, then the therapeutic window for the use of the combination is narrower than what has been assumed.

Previous studies of the interaction between barbiturates and opioids revealed a wide range of patterns. Barbiturates in sub-anesthetic doses antagonize the analgesic effect of opioids; this occurs only when small doses are used /3/. In contrast, fentanyl and thiopental have been found to be synergistic in blocking the righting reflex in rats /3/, considered equivalent to hypnosis in humans, whereas interaction for the suppression of purposeful movement response to noxious stimulus has been found to be infra-additive (relative antagonistic) /2/. On still another level, thiopental does not prevent the bronchoconstriction induced by fentanyl /5/. Nevertheless, an increase in heart rate has been noted in patients anesthetized with thiopental in combination with fentanyl /11/. These interactions, occurring mostly at the pharmacodynamic level, have also been attributed to some extent to pharmacokinetic interactions. In this regard, therapeutic concentrations of fentanyl displace thiopental from albumin and increase its unbound fraction by less than 5%. Some authors have found a correlation between increased unbound fraction of thiopental and shortened interval for induction of anesthesia and prolonged sleep /12/.

Admittedly, our experimental model used non-ventilated animals, whereas in human patients ventilation can be supported mechanically. Nevertheless, our observation that death may occur due to cardiac arrest rather than to respiratory depression should be taken as a warning sign. Although the LD_{50} of thiopental was moderately reduced by an analgesic dose of fentanyl, the steepness of the resultant curve threatens to turn small human errors into major catastrophes.

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