The value of anaesthetic steroids alphaxolone-alphadolone in pregnant mice

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Mortality in mice, during or as a result of anaesthesia, is a well known problem (*Green,* 1982) and this wastage entails an unnecessary increase in the number of laboratory animals used in an experiment.

During the last few years, we have carried out experiments in order to elucidate the genotoxic effect of diagnostic ultrasound in pregnant mice, for which it was necessary to anaesthetize the animals, with a low risk of failure in both the dams and fetuses.

Pregnant mice (NMRI-Bom), at day 15-18 of gestation, were anaesthetized i.v. and placed in a 36°C waterbath, where the ultrasound scanning took place. The scanning-time was from 15 to 45 minutes, whereafter the mice were allowed to recover. The next day the animals were sacrified, the fetuses taken out and blood-cells from the fetal liver were further processed in the transplacental micronucleus test as described by *Cole et al.* (1981).

Until now, we have evaluated four different anaesthetic agents in the following order: Thiopentone (Leopental®), pentobarbitone (Nembutal®), diazepam (Apozepam®) and alphaxolone-alphadolone (Saffan®). Thiopentone and pentobarbitone are barbiturates, which are among the most commonly used anaesthetics in mice. From other studies (*Au et al.* 1982 and *O'Brien* 1976), where mice have been ultrasound scanned, it appears that barbiturates have been used without side-effects, and this was the reason why we also from the beginning decided to use them. The benzodiazepine, diazepam, was used only because we had easy access to it, and because we thought it was a mild agent. Saffan®, a mixture of alphaxolone (0.9% w/v) and alphadolone acetate (0.3% w/v), is the most recent anaesthetic we have tried.

Table 1. Incidence of mortality in pregnant mice using different kind of anaesthetics, i.v.

Anaesthetic	dose mg/kg	number of mice	number of dead mice
Thiopentone	75-100	60	9 (15%)
Pentobarbitone	50-60	60	8 (13%)
Diazepam	25-35	12	1 (8%)*
Alphaxolone-alphadolone	25-**	111	0

*) In 6 of the pregnant mice all fetuses were dead 20 hours after injection, and in one of the mice 5 out of 13 fetuses had died.

**) Initial dose.

Table 1 shows the mortalities in mice within 20 hours of i.v. injection of the four different anaesthetics. The incidence of mortality was highest for the two barbiturates, thiopentone 15%, pentobarbitone 13%. The mortality of the dams was less using diazepam (8%) but this drug had a disastrous effect in the fetuses. With alphaxolone-alphadolone we have never seen any dead mice until now, and neither have we observed any side-effects as we have with the other three agents. Besides the high mortality, the barbiturates gave rise to a slow awakening with the mice in a bad condition during this process, which in our opinion causes unnecessary suffering to the animals. Even worse were the sideeffects of diazepam. The awakening was slower than for the barbiturate anaesthetized mice, and the condition of the animals can only be described as terrible. It is not possible to say whether the suffering was due to the apozepam itself or was a consequence of the toxic effect on the fetuses, which all died in 6 out of 12 mice.

With alphaxolone-alphadolone we have seen no side-effects. Shortly after termination of the anaesthesia, the mice awoke and appeared to recover completely within minutes. A single

dose of alphaxolone-alphadolone (25 mg/kg, i.v.) will anaesthetize the mice for about 10 minutes, and for a longer duration it is merely necessary to inject more anaesthetic. For this purpose we have, without any problems, used a winged infusion set inserted i.v. into a lateral tail vein and this allowed us to give further injections during the experiment whenever the mice showed any sign of awakening. The total dose for 30 minutes anaesthesia was about 50 mg/kg.

Our findings are in agreement with results from studies to determine the anaesthetic potency and acute toxicity of alphaxolone-alphadolone. *Al-Khawashki et al.* (1979) found that an i.v. dose of these steroids ranging from 2-24 mg/kg induced immediate sleep in mice for periods of 4-17 minutes in direct proportionality to the dose level, and the therapeutic index was calculated to be 22.7 (LD50 = 47 mg/kg and AD50 = 2.07 mg/kg). *Child et al.* (1971) found the therapeutic index of the steroids to be 30.6 (LD50 = 54.7 mg/kg and AD50 = 1.7 mg/kg) in mice, when given i.v.. In another study, *Green et al.* (1978) reported the value of alphaxolone-alphadolone if given i.v. to mice, rats, hamsters and neonatal pigs. Further, *Green* (1982) emphasizes that the wide therapeutic index of alphaxolone-alphadolone anaesthetic makes it an excellent agent for mice. The short period of anaesthesia can easily be extended by using the winged infusion set as mentioned earlier, and making this extra effort is nothing compared to the satisfaction of knowing that the animals, when anaesthetized with alphaxolone-alphadolone are completely unconscious, feel no pain, are fully relaxed and recover rapidly at the end of the experiment. Of particular importance to us was the knowledge that the fetuses also survived the anaesthesia.

Summary

Thiopentone, pentobarbitone, diazepam and alphaxolone-alphadolone were evaluated as anaesthetic agents in pregnant mice. Incidence of mortality was: Thiopentone 15%, pentobarbitone 13%, diazepam 8%, and alphaxolone-alphadolone 0%. Besides the low mortality, alphaxolone-alphadolone also distinguished by showing no side-effects at all, neither to the dams nor to the fetuses, and it is recommended as an excellent anaesthetic in pregnant mice.

Sammendrag

Thiopentone, pentobarbitone, diazepam og alphaxolone-alphadolone er anvendt som anæstesimidler i gravide mus. Forekomsten af dødsfald blandt dyrene var: Thiopentone 15%, pentobarbitone 13%, diazepam 8% og alphaxolone-alphadolone 0%. Foruden at have den laveste forekomst af dødsfald, udmærkede alphaxolone-alphadolone sig desuden ved ikke at vise tegn på bivirkninger, hverken i moderdyr eller fostre, og det anbefales som et glimrende anæstheticum til gravide mus.

Yhteenveto / K. Pelkonen

Työssä arvioitiin tiopentonia, pentobarbitonia, diatsepaamia ja alphaxolone-alphadolonea nukutusaineena kantaville hiirille. Kuolemantapauksia sattui tiopentonilla 15%, pentobarbitonilla 13%, diatsepaamilla 8% ja alphaxolone-alphadolonella 0%. Viimemainitulla ei myöskään havaittu mitään sivuvaikutuksia, ei emoihin eikä sikiöihin. Tekijät suosittelevat alphaxolone-alphadolonea erinomaisena anesteettina kantaville hiirille.

References

Al-Khawashki, M. I., H. A. Galeb, N. El-Gawhary, M. K. Madkour, A. M. Radwan & A. M. El-Sherbiny: Pharmacological assessment of the anaesthetic potency and acute toxicity of Althesin and its steroidal components in laboratory animals. *J. Egypt. Med. Assoc.*, 1979, *62,* 191-208.

Au, W. W., N. Obergoenner, K. L. Goldenthal, P. M. Corry & V. Willingham: Sister-chromatid exchanges in mouse embryos after exposure to ultrasound in utero. *Mut. Res.*, 1982, *103,* 315-320.

Child, K. J., J. P. Currie, B. Davis, M. G. Dodds, D. R. Pearce & D. J. Twissell: The pharmacological properties in animals of CT1341 – a new steroid anaesthetic agent. *Brit. J. Anaesth.*, 1971, *43,* 2-13.

Cole, R. J., N. Taylor, J. Cole & C. F. Arlett: Short-term tests for transplacental active carcinogens. I. Micronucleus formation in fetal and maternal mouse erythroblasts. *Mut. Res.*, 1981, *80,* 141-157.

Green, C. J., M. J. Halsey, S. Precious & B. Wardley-Smith: Alphaxolone-alphadolone anaesthesia in laboratory animals. *Lab. Anim.*, 1978, *12,* 85-89.

Green, C. J.: Animal Anaesthesia. Laboratory Animal Handbooks 8. London, 1982, pp 147-154.

O'Brien, W. D.: Ultrasound induced fetal weight reduction in mice. *Ultras. Med.*, 1976, *2,* 531-532.