Does preoperative administration of metamizol (Novalgin®) affect postoperative body weight and duration of recovery from ketamine xylazine anaesthesia in mice undergoing embryo transfer: A preliminary report

M. M. Dorsch, K. Otto & H. J. Hedrich

Institute for Laboratory Animal Science, Medical School Hanover, Carl-Neuberg Strasse 1, 30625 Hannover, Germany

Summary

Thirty-two female mice used for embryo transfer or as controls received either metamizol or equal volumes of normal saline administered subcutaneously following induction of anaesthesia with ketamine–xylazine. Body weight was measured immediately before surgery, after 24 and after 48 h. The duration of the surgical anaesthesia was recorded and postoperative behavioural responses were measured. Comparison of the treatment groups revealed no significant differences in body weight and recovery times, nor were other signs of discomfort detected in either treatment group. It was concluded that administration of metamizol did not provide additional analgesia following embryo transfer in mice anaesthetized with ketamine–xylazine.

Keywords Metamizol; ketamine–xylazine; anaesthesia; mouse; embryo transfer

The transfer of embryos is an important step for the rederivation of cryopreserved animal stocks and for the generation of genetically modified mice. After the manipulation of the embryo either by microinjection of DNA into the pronucleus of fertilized eggs, or after injection of genetically modified embryonic stem cells into blastocysts, these embryos have to be transferred to the reproductive tract of surrogate dams to complete their development. This needs invasive surgery and anaesthesia that lasts for at least 20 min. A combination of ketamine and xylazine is one of the most commonly used injectable anaesthesia techniques in rodents. Although both ketamine and xylazine exert analgesic effects by acting on N-methyl-D-aspartate (NMDA) (Maurset *et al*. 1989) and adrenergic alpha2-receptors (Maze & Tranquilli 1991) respectively, additional postoperative pain relief in rats has been recommended before (Liles & Flecknell 1994, Flecknell *et al*. 1999).

Since mild to moderate pain intensities may be expected following laparotomy (Crane 1987), non-steroidal anti-inflammatory drugs (NSAIDs) may be a suitable choice for pain relief (Thurmon *et al.* 1996). Metamizol (Dipyrone) is a pyrazolon

Correspondence to: M. M. Dorsch
E-mail: dorsch.martina@mh-hannover.de

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derivative with analgesic, spasmolytic, antiphlogistic and antipyretic properties (Frey *et al*. 1996). The analgesic effects of dipyrone administered at 200 mg/kg body weight subcutaneously to rats have been reported (Abbot & Bonder 1997).

A number of clinical variables including food and water intake, body weight, and duration of recovery from anaesthesia have been suggested for the assessment of postoperative pain particularly in laboratory rodents (Morton & Griffiths 1985, Soma 1985, Liles & Flecknell 1994, Flecknell *et al.* 1999). In previous studies, food and water consumption and body weight were depressed following surgical procedures due to postoperative pain (Flecknell & Liles 1991, Liles & Flecknell 1993, Wolfensohn & Lloyd 1995), the degree of depression could be reduced by adequate postoperative pain relief (Liles & Flecknell 1994, Flecknell *et al*. 1999). In addition, a recovery period of an unusual length of time has been suggested as an indication for postoperative pain (Soma 1985).

Most studies in this area were done on rats. However, little is known about pain relief in mice undergoing surgery. Therefore, the objective of the present study was to assess the effects of metamizol on postoperative body weight, recovery from anaesthesia, and some behavioural patterns in mice submitted to embryo transfer.

Material and methods

Animals

Thirty-two F1 hybrid mice (Ztm:NMRI × C57BL/6JZtm) were used as surrogate dams for the transfer of 2-cell embryos (freeze-thawed from a genetic repository to rederive mouse inbred strains) or as controls without surgery. Mating the females to vasectomized males with proven sterility (1:1) induced pseudopregnancy. Pseudopregnancy was assumed when a vaginal plug was visible in the morning after mating (day 0.5).

Husbandry

The animals used for this study were housed under conventional conditions at the Central Animal Facility of the Medical School Hannover, Germany (laboratory code: Ztm). The animal rooms had a controlled environment: 21 ± 2°C, 50 ± 5% relative humidity with artificial light from 07:00 to 19:00 h. The mice were acclimatized to this environment from birth. They were fed a commercial pelleted diet (Altromin 1314, Altromin, Lage, Germany) containing 22% protein, 5% fat, 4.5% raw fibre, and 7% ash, utilizing energy was 3.1 kcal/g. The animals were caged in Makrolon® type II cages (Techniplast, Italy) on bedding of dust-free softwood granulate (Linocel, Altromin), tap water was available *ad libitum*. The vasectomized males were caged individually, except for the production of pseudopregnant females. The females were caged as groups of four animals, except for the induction of pseudopregnancy. After embryo transfer they were caged as groups again and not separated until shortly before parturition.

For embryo transfer, pseudopregnant females were transferred to the surgery room at 08:00 h. They were not returned to the animal room until their recovery from anaesthesia.

Anaesthesia

For anaesthesia, a freshly prepared mixture of 0.5 ml ketamine HCl 10% (WDT, Garbsen, Germany) and 0.1 ml xylazine HCl 2% (Rompun®, Bayer, Leverkusen, Germany) diluted with sterile saline solution (Merck, Darmstadt, Germany) to a total volume of 5.0 ml was used.

The mice were anaesthetized by intraperitoneal (i.p.) injection of 0.1 ml of the mixture per 10 g body weight (bwt) equal to a dose of 100 mg/kg ketamine and 4 mg/kg xylazine. Dexpanthenol eye ointment (Bepanthen®, Hoffmann-La Roche, Leverkusen, Germany) was administered for corneal protection. In order to prevent hypothermia, cages with anaesthetized mice were placed on a warming plate (25–30°C) until recovery.

Treatment groups

The animals were assigned to the following four treatment groups ($n = 8$). At least one

animal per treatment group was measured on one day. All experimental measurements were performed by the same staff.

- Group 1: no embryo transfer (nETS)—Anaesthesia as described plus subcutaneous injection of 0.1 ml/100 g bwt saline.
- Group 2: no embryo transfer (nETN)—Anaesthesia as described plus subcutaneous injection of 200 mg/kg bwt metamizol (Novalgin®, Hoechst Veterinär, Germany), diluted in saline (20 mg/1 ml saline).
- Group 3: embryo transfer (ETS)—Anaesthesia plus subcutaneous injection of saline as described. Embryo transfer was carried out as described (Hogan *et al.* 1994).
- Group 4: embryo transfer (ETN)—Anaesthesia plus subcutaneous injection of metamizol as described. Embryo transfer was carried out as described. Saline and metamizol were applied by subcutaneous injections at the dorsal aspect of the neck as soon as toe pinch reflex (forceps) disappeared.

Variables

(1) The body weight was measured immediately before surgery, and 24 h and 48 h later.
(2) Duration of surgical anaesthesia: starting with the moment of injection of the anaesthetic agents until re-appearance of the toe pinch reflex.
(3) Postoperative behavioural responses were measured as soon as the toe pinch reflex returned and then every 15 min for 3 min each. Last measures for each mouse were recorded 135 min after return of the toe pinch reflex. At that time nearly all females showed more or less normal behaviour. The behavioural pattern was according to a description by van Loo *et al.* (1997).

Gait: the mouse is running or walking through the cage, using the whole available space:

score 1: normal locomotion, ascertaining the cage
score 2: reduced locomotion
score 3: lethargic behaviour
score 4: listless, sitting in one corner of the cage

Grooming behaviour: the mouse shakes its fur, or is stretching. It is wiping or licking its fur, ears, tail or genitals:

score 1: normal grooming behaviour
score 2: reduced grooming behaviour
score 3: lethargic behaviour
score 4: listless, sitting in one corner of the cage

Appearance of the coat:

score 1: normal, shining
score 2: moderate change
score 3: fur begins to stand (piloerection), loses shining
score 4: severe change

A distress scoring sheet as suggested by Wolfensohn and Lloyd (1995) was drawn for each female as shown in Table 1. The overall score indicates the likelihood of whether the animal is suffering.

Table 1 Distress scoring sheet

Animal identification: Treatment group:

	Score								
Behaviour	15 min	30 min	45 min	60 min	75 min	90 min	105 min	120 min	135 min
Gait									
Grooming									
Coat									
Total	3 12								

Judgement: 3 5 normal; 6 8 slight distress; 9 11 suffering; 12 severe pain

Statistical analyses

The StatView 5.0 program (http://www.statview.com) was used for statistical analysis. The pre- and postoperative body weight and the duration of the surgical anaesthesia were compared using Bonferroni/Dunn and Students's *t*-test. The total scoring for each time point and for each female was compared using the Bonferroni/Dunn test. Significance levels were set at $P \leq 0.05$.

Results

Duration of anaesthesia

Table 2 summarizes the mean periods of time for the duration of anaesthesia. There were no significant differences between the treatment groups as shown by the Bonferroni/Dunn and Students's *t*-test. There were individual differences in the sleeping period. Minimum sleeping time of treatment group 1 (anaesthesia plus saline: nETS) was 45 min, maximum 120 min. Sleeping time of treatment group 2 (anaesthesia plus metamizol: nETN) varied from 55–75 min, treatment group 3 (embryo transfer plus saline: ETS) varied from 49–120 min and treatment group 4 (embryo transfer plus metamizol: ETN) ranged from 45–90 min.

Table 2 Duration of anaesthesia

Treatment group	Duration (min) ± SE
nETS	65.25 ± 2.89
nETN	76.25 ± 9.77
ETS	75.00 ± 6.55
ETN	73.13 ± 5.97

Duration of anaesthesia was the time from injection until return of toe reflex. Comparison with Bonferroni/Dunn showed no significant differences between treatment groups ($P < 0.05$). nETS = anaesthesia plus saline; nETN = anaesthesia plus metamizol; ETS = embryo transfer plus saline; ETN = embryo transfer plus metamizol

Body weight

The body weights of the four treatment groups are represented in Table 3. One sample *t*-test confirmed that the pre- and postoperative body weights were consistent within the different treatment groups. Comparison of the pre- and postoperative body weights of the different treatment groups with Bonferroni/Dunn showed no significant difference. All animals reached their initial body weight at least 48 h after starting the treatment, regardless of whether or not they received analgesia or underwent surgery.

Behaviour

Figure 1 shows the results of the overall score for each treatment group over a time period of 135 min from re-appearance of the toe pinch reflexes. 'Abnormal' behaviour, such as reduced locomotion or lethargic behaviour, shortly after re-appearance of the toe pinch reflex was also observed in those treatment groups that did not undergo embryo transfer, leading to the conclusion that this behaviour was due more to the anaesthesia than to the surgery. The overall scores for every animal were compared using Bonferroni/Dunn at each time point. No significant differences were observed for any time point. However, the scores for the treatment groups that underwent embryo transfer (5.6 ± 0.6 for ETS; 4.6 ± 0.6 for ETN) were slightly higher than those for the groups without surgery (4.3 ± 0.5 for nETS; 3.8 ± 0.5 for nETN) at the end of the observation

Table 3 Body weight (g) ± SE

Treatment group	Pre-treatment	24 h later	48 h later
nETS	23.88 ± 1.39	24.00 ± 1.59	24.38 ± 1.66
nETN	26.00 ± 1.51	25.14 ± 1.50	25.57 ± 1.49
ETS	23.71 ± 1.80	23.57 ± 0.97	23.86 ± 0.96
ETN	24.00 ± 0.46	23.75 ± 0.70	24.75 ± 0.67

Comparison with Bonferroni/Dunn showed no significant differences between treatment groups ($P < 0.05$). See Table 2 for abbreviations

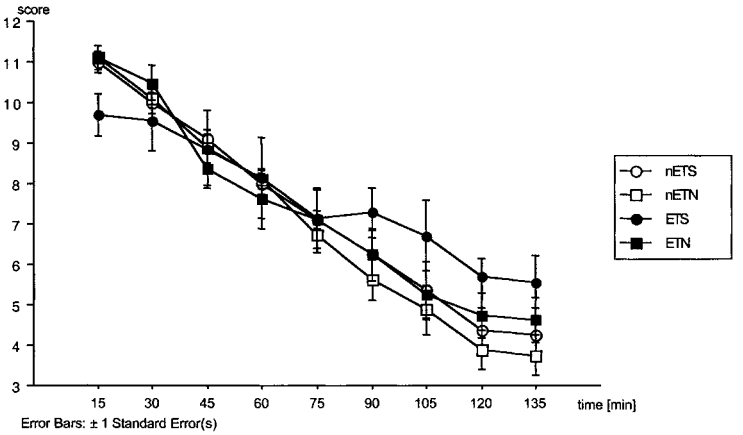

Fig 1 Overall score for behavioural patterns observed after recovery of the toe pinch reflex. nETS: application of anaesthesia plus saline without embryo transfer, nETN: application of anaesthesia plus Novalgin®, ETS: embryo transfer under anaesthesia plus application of saline, ETN: embryo transfer under anaesthesia plus application of Novalgin®. Judgement of the score: 3 5: normal behaviour, 6 8: slight distress, 9 11: suffering, 12: severe pain

time, indicating moderate distress for the mice that underwent surgery. Comparison of the 135 min data may also indicate a slight, yet not significant, positive effect of metamizol.

Discussion

In the present study, the effects of subcutaneous administration of either metamizol or saline on changes in body weight and well-being following embryo transfer in mice were evaluated. This was undertaken to determine the need for an analgesic agent in addition to ketamine–xylazine anaesthesia.

All treatment groups had a slightly reduced body weight 24 h after embryo transfer, except the group that received anaesthesia plus saline (nETS). All animals reached their initial or an increased body weight at least 48 h after beginning the treatments. This suggests that animals continued to drink and eat following recovery from the anaesthesia. Since additional treatment with metamizol was not associated with a higher increase in postoperative body weight, we assume that ketamine and xylazine used for anaesthesia may have provided adequate postoperative analgesia (Maurset *et al.* 1989, Maze & Tranquilli 1991) with respect to the type of surgery.

Postoperative pain may also be indicated by an unusual length of recovery time (Soma 1985). The duration of surgical anaesthesia in our study was similar for all treatment groups, again, suggesting adequate pain relief by ketamine–xylazine alone.

This assumption is further supported by the fact that neither group of mice showed behavioural signs suggesting postoperative discomfort such as aggression or vocalization upon manipulation (Morton & Griffiths 1985). For the validation of this assumption we monitored some typical behavioural patterns using a distress scoring (as suggested by Wolfensohn & Lloyd 1995) for decrease or increase in activity (gait), poor grooming habits, and piloerection for more than 2 h after surgery. All animals had more or less normal behavioural patterns after the observation time.

Based on the type of surgery and with the techniques currently available for assessing

postoperative pain in mice we conclude that administration of metamizol at 200 mg/kg subcutaneously did not provide significant additional analgesia following embryo transfer in mice. However, as the score for the group undergoing surgery without additional analgesia (ETS) was slightly increased, as compared to the other groups, especially to the group undergoing surgery with additional analgesia (ETN), further studies including the use of more potent analgesic agents and the employment of additional clinical signs of pain will be needed to support this conclusion. Furthermore, the type of NSAID (metamizol) used and/or the single dose of 200 mg/kg s.c. tested in our study may have been inadequate in producing a significant difference to the control group.

Further studies on the analgesic effects of other NSAIDs in laboratory rodents are needed, because the majority of opioid analgesics are controlled drugs, thus limiting their use.

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