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Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Evaluation of anesthesia and reproductive performance upon diazepam and xylazine injection in rats

Abd El-Nasser A. Mohammed[1]*, Mahmoud A. Abdelnabi[1], Jacek A. Modlinski[2]

[1] Department of Animal and Poultry Production, Faculty of Agriculture, University of Assiut, Assiut 71526, Egypt

[2] Department of Experimental Embryology, Polish Academy of Sciences Institute of Genetics and Animal.Breeding, Jastrzebiec, 05-552 Magdalenka, Poland

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The aims of the current study were to evaluate the effects of diazepam (D) and/or xylazine (X) on the induction of sedation and anesthesia, performing ovarian transplantation and litter size upon administration during pregnancy. One hundred and five albino rat females were classified randomly into four groups given intraperitoneal (IP) injection of physiological saline (group I), diazepam (6.2 mg /kg body weight - group II), xylazine (13.2 mg/kg/body weight - group III) and 6.2 mg/kg body weight diazepam and 13.2 mg/kg body weight xylazine (group IV). Induction of sedation and anesthesia were monitored. The onset of anesthesia, duration of surgical anesthesia and sleep time after injections was recorded. Rectal temperatures were monitored at 0, 20, 40, 60 and 120 min after injection. Blood samples were collected at 0 and 120 min post injection for determination of hemoglobin, glucose and urea concentrations. Ovarian transplantation was performed in females anesthesized with DX drugs. Mated females were injected with physiological saline, 6.2 mg/kg diazepam and/or 13.2 mg/kg xylazine at day 4, 11 and 18 of pregnancy and litter sizes were recorded. Sedation was obtained after injection of diazepam or xylazine alone whereas surgical anesthesia was obtained upon injection of both diazepam and xylazine. The onset of anesthesia, duration of anesthesia and sleep time after DX injection was 4.6, 144.1 and 79.1 min, respectively. Rectal temperature decreased (P<0.05) after injection of diazepam and/or xylazine. The hemoglobin

*Corresponding author: elnasser@aun.edu.eg

(P<0.05), glucose and urea blood concentrations increased after injection of diazepam and/or xylazine. Upon ovarian transplantation, all animals recovered and ovarian follicles were found in the transplanted ovaries. Number of offspring per animal did not differ due to diazepam and/or xylazine injection at day 4, 11 and 18 of pregnancy. In conclusion, DX injection induced surgical anesthesia in rats sufficient for performing major surgeries.

KEY WORDS: anesthesia / diazepam /ovarian follicles / xylazine

Many animal models used in research must be anesthesized and/or surgically prepared for investigations. The proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative. Although the use of anesthetics and analgesics for sample collection and minor/major surgical interventions is becoming more common in veterinary and scientific practices, caution must be taken as some of these drugs can cause serious irreversible injuries to cardiovascular and pulmonary organs [Borkowski *et al*. 1990, Flecknell *et al*. 1983, Peeters *et al*. 1988] and interact with the aim of the study.

Diazepam or xylazine were used for sedation induction in mammals but they cause reversible/irreversible side effects [Yadav 2008, Ghurashi *et al*. 2009, Mohammed *et al*. 2011]. Diazepam or xylazine were reported to affect the respiratory system, heart rate and rectal temperature [Bright 1986, Hall *et al*. 2001, Yadav 2008]. Hematological and biochemical changes were found during diazepam or xylazine administration [Fani *et al*. 2008]. In addition, an increased risk of congenital malformations associated with the use of minor tranquilizers (diazepam) during the first trimester of pregnancy has been suggested in several studies (http://www. patientsville.com/labels /diazepam _label.htm). On the other hand, the changes in uterine contractility and fetal heart rate caused by a single injection of 20 mg xylazine in heifers in the first trimester of gestation have no short-term adverse effects on pregnancy (Dobrinski *et al*. 1994]. In pregnant cows during late gestation, xylazine markedly reduces flow and availability of oxygenated blood to the uterus, which may critically impair delivery of oxygen to the fetus at a stressful and important time of development or delivery [Hodgson *et al*. 2002].

Diazepam and xylazine are sedative pre-anesthetic medication with concentration 2.5-5 and 1-5 mg/kg body weight, respectively [Flecknell 2003] Diazepam was reported to cause good muscle relaxation and can be used to cure convulsions [Averill 1970] while xylazine has myorelaxant and potential local anesthetic properties. Because diazepam or xylazine drugs have a dose-dependent effect [Muir and Mason 1993] evaluation of their dose on surgical anesthesia induction was performed in rats. Moreover, the quality of surgical anesthesia was evaluated through ovarian transplantation operation upon DX anesthesia. Therefore, the aim of the present study was to investigate the effects of diazepam (D) and/or xylazine (X) drugs on the induction of sedation and surgical anesthesia, performing ovarian transplantation and litter size upon drugs' administration during pregnancy.

Material and methods

Animals and experimental groups. Female rats (300-350 g body weight) were used during the 2.5 month study and classified into four groups. The control group was given intraperitoneally (IP) 0.7 ml physiological saline and the rats of remaining three groups IP 6.2 mg diazepam and/or 13.2 mg xylazine/kg body weight. Rats were obtained from the house of lab animals, the Faculty of Human Medicine, University of Assiut. All animals had free access to a commercial diet and fresh water and the appropriate animal care protocol was followed throughout the experimental period. The optimum diazepam and xylazine anesthesia levels were chosen through preliminary investigations.

Experiment I

A total of forty albino rats of 300-350g body weight were randomly allotted to four groups, ten animals each. The first group was injected with 0.7 ml physiological saline. The other three groups were injected with 6.2 mgkg diazepam (Neuril 2.5 mg/kg BW; Memphis Co. for Pharm & Chemical Ind., Cairo, Egypt) or 13.2 mg/kg BW xylazine (Xylaject 10 mg/kg; ADWIA Co. S. A. E. 10th of Ramadan city, Cairo, Egypt) or their combinations (DX). Rectal temperatures (RT) were recorded at 0, 10, 20, 40, 60 and 120 min post injection. Blood samples were collected at 0 and 120 min after the injection to determine of hemoglobin, glucose and urea. Induction of sedation and anesthesia was monitored by the animal's reflexes in response to pedal pinching.

Induction of sedation and surgical anesthesia. The animals were given IP injection of 6.2 mg diazepam and/or 13.2 mg xylazine/kg BW. The induction and maintenance of sedation and general anesthesia were assessed according to the rat's responsiveness to a painful stimulus (pedal reflex). The sedation was ranked to the response of animal to pedal reflex. In the case of surgical anesthesia, the time from drug administration till the animal starts gaining consciousness was classified into three periods: onset of anesthesia (times to loss the righting reflex), duration of anesthesia (indicated by loss of the pedal reflex) and sleep time (recovery of the righting reflex).

Blood sampling. Blood samples were collected from ten animals in each group at 0 and 120 min from injection with 6.2 mg/kg BW diazepam and/or 13.2 mg/kg BW xylazine. Samples were collected from the orbital sinus according to Hoff [2000]. Hemoglobin concentrations were determined immediately. Then, the remaining blood was centrifuged at 5000 rpm for 15 min to obtain plasma and stored at -20 ˚C until further analysis for glucose and urea.

Blood plasma analysis. Plasma was analysed for glucose and urea using commercial test kits supplied by Spectrum Diagnostics (Cairo, Egypt). The concentrations were measured using standard protocols.

Experiment II

Ovarian transplantation. Five females were anesthesized with 6.2 mg diazepam/kg BW and 13.2 mg/kg BW of xylazine. Ovarian transplantation was performed as previously described [Dorsch *et al*. 2004]. Briefly, a single transverse incision of the skin at the right dorsal, across the lumbar area, gave access to the right ovary on right side. A small slit was made in the fat surrounding the ovarian bursa to expose the ovary. The recipient's ovarian tissues were removed and transplanted into the respective ovarian bursa. Then, the small slit in the fat surrounding the ovarian bursa was closed. The ovarian complex was replaced in the body cavity, and the incision was closed. Eight weeks after ovary transplantation, the recipient females were killed by cervical dislocation and the ovarian tissues were investigated through visible follicle numbers under microscope.

Experiment III

Litter size upon diazepam and/or xylazine injection during pregnancy. Sixty mated females were classified into four groups. They were injected with physiological saline with 6.2 mg diazepam per kg BW and/or 13.2 mg/kg BW xylazine at day 4, 11 and 18 of pregnancy. Litter sizes were recorded.

Statistical. Data are presented as means ± SD. Differences between mean values were evaluated by ANOVA followed by comparisons using the Duncan's multiple range test. Differences with $P < 0.05$ were considered significant.

Results and discussion

Results of the current study demonstrated that a combination of DX can be used for induction of surgical anesthesia characterized by smooth recovery and of sufficient duration and depth for performing ovarian transplantation in rats without serious impairment of their vital functions. Although transient negative side-effects such as hypothermia were observed following drug administration, D and/or X administration on day 4, 11 and 18 did not affect the reproductive performance defined by the litter size.

Experiment I

1. Effect of diazepam and/or xylazine on induction of sedation and anesthesia. Sedation was obtained after injection of 6.2 mg/kg BW diazepam or 13.2 mg/kg BW xylazine alone. Xylazine caused light to moderate sedation, little to no analgesia. On the other hand, diazepam caused light sedation and no analgesia. Surgical anesthesia was obtained upon injection of both 6.2 mg/kg BW diazepam and 13.2 mg/kg BW xylazine. The onset of anesthesia, duration of surgical anesthesia and sleep time after injection wee 4.6, 144.1 and 79.1 min, respectively. In this study, diazepam and xylazine were given intraperitoneally (IP) that is better than intramuscular (IM) because it causes less pain and reduces stress during induction. Diazepam and

xylazine are sedative pre-anesthetic medication with concentration 2.5-5 and 1-5 mg/kg BW [Flecknell 2003]. Therefore, increasing the concentrations to 6.2 mg/kg BW of diazepam and 13.2 mg/kg BW of xylazine resulted in general anesthesia. There are different anesthesia injectable drugs used in rats as ketamine-xylazine and ketamine-diazepam combinations. Such combinations gave 20-30 min duration of anesthesia which is too short for complex surgical operation. Prolongation of surgical anesthesia upon DX administration might be due to diazepam drug. Diazepam may be appropriate for long-term sedation [Jacobi *et al*. 2002]. To the best of our knowledge, diazepam and xylazine combination was not tested so far as anesthetic drugs in rats. Therefore, rectal temperatures, blood parameters and reproductive performance were monitored upon drugs' administration.

2. Effect of diazepam and/or xylazine on rectal temperature. Rectal temperature gradually decreased ($P<0.05$) upon injection with 6.2 mg/kg BW diazepam and/or 13.2 mg/kg xylazine compared to control till 120 min of injection (Tab. 1, Fig. 1). The reduction in rectal temperature was more marked ($P<0.05$) with xylazine or

Table 1. Effect of injections of 6.2 mg diazepam and/or 13.2 mg xylazine (mg per kg body weight) on rectal temperature (RT) in rats

Item	Time (min)	Control	Diazepam(D)	Xylazine (X)	D & X
RT	0	36.7±0.2	36.65±0.2	36.61±0.2	36.65±0.4
	20	36.72^{a}±0.2	36.23^{b}±0.2	35.11^{c}±0.7	35.18^{c}±0.5
	40	36.67^{a}±0.3	35.60^{b}±0.3	34.3^{c}±0.4	33.78^{d}±0.5
	60	36.72^{a}±0.3	35.18^{b}±0.5	33.97^{c}±0.3	33.82^{c}±0.5
	120	36.76^{a}±0.2	33.95^{b}±0.8	31.09^{c}±1.0	30.8^{c}±1.0

^{abcd}In the same row values with different superscripts differ significantly at $P<0.05$.

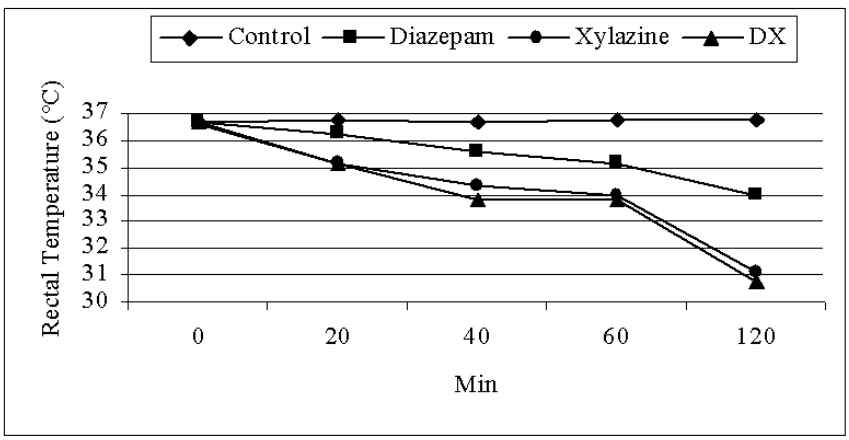

Fig. 1. Effect of 6.2 mg diazepam and/or 13.2 mg xylazine injections (mg per kg body weight) on rectal temperature in rats.

diazepam and xylazine combination compared to diazepam injection alone. Such trend occurred upon D and/or X administration to cattle byYadav [2008], male Mongolian gerbils [Sarnowska *et al*. 2009**]** and rabbits (Mohammed *et al*. 2011, submitted for publication). Therefore, thermoregulatory control is changed upon diazepam and/or xylazine administration in rats. The animals become more susceptible to hypothermia during the sedative and anesthetic effects of the drugs.

3. Effect of diazepam and/or xylazine on blood components. The hemoglobin, glucose and urea concentration increased after two hours of 6.2 mg/kg BW diazepam and/or 13.2 mg/kg BW xylazine injection (Tab. 2). These results are in accordance with observations made by Symonds [1976] on cattle, Custer *et al*. [1977] on camels, Dwivedi and Sharma [2004] on buffaloes and Fani *et al*. [2008] on dogs upon drugs' administration. The increase in hemoglobin concentration might be due to sequestration of blood cells in spleen and lungs during anaesthesia [Lumb and Jones, 1997]. Hyperglycemia might be due to the stress-induced gluconeogenesis as a result of anesthesia and probable suppression of insulin and increased production of glucose in the liver. The mean value for glucose obtained during xylazine-induced restraint was approximately twice that found in manually restrained camels **[**Custer *et al.* 1977]. The elevation of blood urea nitrogen is attributed to the temporary inhibitory effects of drugs on renal blood flow [Fani *et al*. 2008] which in turn might have caused a rise in blood urea nitrogen.

Table 2. Effect of injections of 6.2 mg diazepam and/or 13.2 mg xylazine (mg per kg body weight) on blood hemoglobin and blood plasma glucose and urea concentrations in rats

Item	Time (min)	Control	Diazepam(D)	Xylazine (X)	D & X
Hb	0	12.1±1.8	12.1±1.7	12.3±2.0	12.07±1.0
	120	12.2^{b}±1.7	13.8^{a}±0.55	14.0^{a}±1.2	14.06^{a}±0.5
Glucose	0	103.4±5.3	103.4±4.9	102.5±	101.0±7.4
	120	103.1±5.1	106.2±7.4	106.4±9.4	107.8±12.8
Urea	0	54.9±9.6	55.5±9.3	55.8±12.6	55.00±8.0
	120	56.1±9.6	57.2±8.7	58.3±8.8	59.00±4.3

^{ab}In the same row values with different superscripts differ significantly at $P<0.05$.

Experiment II

Effect of diazepam and/or xylazine on the quality of surgical anesthesia. The use of DX combination produced general anesthesia which was deep and long enough (144.1 min) to accommodate ovarian transplantation. The duration required for performing surgical procedures averaged 35 min. Surgeries were conducted without serious complications. Hypothermia observed during surgical anesthesia returned to baseline value and the recovery was smooth. After six weeks of ovarian transplantation, the numbers of follicles per transplanted ovary were 16.3±1.5. Therefore, protocol of DX anesthesia can be used for reproductive studies.

Experiment III

Effect of diazepam and/or xylazine injection during pregnancy on litter size. Number of offspring per animal was not differentiated due to 6.2 mg/kg BW diazepam and/or 13.2 mg/kg BW xylazine injected on day 4, 11 and 18 of pregnancy (Tab. 3). This indicated that D and/or X administration results did not affect the reproductive performance in accordance with the earlier results. A single injection of 20 mg xylazine into heifers in the first trimester of gestation had no short-term adverse effects on pregnancy [Dobrinski *et al*. 1994]. Doses of 1, 2, 5 and 8 mg diazepam/kg BW from day 6 through day 18 of gestation exhibited no adverse effects on reproduction and no teratological changes were identified in rabbits (http://www.patientsville.com/labels/diazepam_ label.htm). Van Geijn *et al*. [1980]) found that duration of the effect of diazepam during pregnancy is dependent on dosage and route of administration.

Table 3. Effect of injections of 6.2 mg diazepam and/or 13.2 mg xylazine (mg per kg body weight) on day 4, 11 and 18 of pregnancy on litter size in rats

Pregnancy	Control	Diazepam (D)	Xylazine (X)	D & X
Day 4	9.0±1.58	8.6±1.14	8.6±2.07	8.4±1.81
Day 11	9.2±1.30	9.0±1.14	8.4±2.40	8.4±1.14
Day 18	9.4±1.14	8.8±1.30	8.8±1.92	8.4±1.14

It could be concluded that administration of 6.2 mg/kg BW of diazepam and/or 13.2 mg/kg BW xylazine can be used to induce sedation and/or general anesthesia during reproductive studies on rats with no adverse effects on pregnancy.

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