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TOPOGRAPHIC AND ANATOMICAL JUSTIFICATION OF THE APPROACH TO PERFORMING INTRAPERITONEAL INJECTION

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Performing intraperitoneal injections in rats is one of the most common and accessible methods of administering drugs and anesthetics in an experiment. In 74 % of cases (32/43), the cecum was located on the right and along the midline. A midline was performed in the laboratory animal from the external opening of the urethra to the xiphoid process. Through the point that divides it in half, a perpendicular was drawn along the left half of the abdomen's front wall. A bisector formed at an angle of 90° to the inguinal fold was lowered into the left inguinal region. The injection site was defined as a point lying on ½ of the line formed by the bisector of the angle. The proposed technical solution has significant advantages: it justifies the place and point of intraperitoneal injection and reduces the risk of iatrogenic damage to abdominal organs.
Key words: intraperitoneal injection, rats, cecum.

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ТОПОГРАФО-АНАТОМІЧНЕ ОБГРУНТУВАННЯ ПІДХОДУ ДО ВИКОНАННЯ ІНТРАПЕРИТОНЕАЛЬНОЇ ІН'ЄКЦІЇ

Виконання інтраперитонеальних ін'єкцій у щурів – один з найбільш поширених та доступних методів введення лікарських препаратів та анестетиків у експерименті. У 74 % випадків (32/43) сліпа кишка розташовувалась справа та по серединній лінії. Лабораторній тварині проводили серединну лінію від зовнішнього отвору уретри до мечоподібного відростка. Через точку, що ділить її навпіл, проводили перпендикуляр по лівій половині передньої стінки живота. У ліву здухвинну ділянку опускали бісектрису, утвореного кута 90° до пахвинної складки. Місце для ін'єкції визначали як точку, що лежить на ½ лінії утвореної бісектрисою кута. Запропоноване технічне рішення має суттєві переваги: обгрунтовує місце та точку виконання інтраперитонеальної ін'єкції та знижує ризик ятрогенного ушкодження органів черевної порожнини.
Ключові слова: інтраперитонеальна ін'єкція, щурі, сліпа кишка.

The study is a fragment of the research project "Development of modern and improvement of existing methods of diagnosis, treatment, prevention and rehabilitation of surgical pathology in children", state registration No. 0123U102436.

The choice of the method of administering the drug to a laboratory animal during the experiment involves the assessment of the main properties of the substance, in particular – sterility, pH, osmolarity, pharmacokinetic features and frequency of administration [7]. Performing intraperitoneal injections in rats is one of the most common and accessible methods of administering drugs and anesthetics in an experiment [2, 9].

Among its advantages over intravenous administration is primarily the ease of manipulation [3] and the absence of the need for catheterization of the lateral tail or iliac veins, the risk of phlebitis or external infection. The vast majority of the injected drug is absorbed into the bloodstream through the mesenteric vessels and passes through the portal vein system, which brings it closer to oral administration [3], but the process is faster, therefore it serves as an affordable alternative for researchers.

The purpose of the study was to improve the technique of performing intraperitoneal injections in sexually mature male Wistar rats and to determine anatomical landmarks for a fixed injection site with minimal risk of complications.

Materials and methods. The study was carried out in the conditions of the research laboratory of the National Pirogov Memorial Medical University, Vinnytsya. The experiment was carried out in accordance with the provisions of the European Convention on the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986) and in accordance with the permission of the Bioethics Committee of National Pirogov Memorial Medical University, Vinnytsya.

The technical component of the study was performed by one operating team throughout the entire period of the experiment. Animals used in the study were sexually mature male rats of the Wistar line, which were subject to general anesthesia, intraperitoneal injection of the drug and primary or repeated laparotomies according to the protocols of the main experiments. The age of the animals was 8.5±1.1 weeks, the body weight was 213.81±10.9 g. The total number of animals was 48.

The study was carried out in 2 stages. At the first stage, 28 animals were used in the acute experiment, which after general anesthesia underwent laparotomy and collection of material for pathomorphological examination. This group of animals was divided into 2 subgroups: No. 1 – 14 animals received an injection of anesthetic in the right iliac region, No. 2 – 14 animals – in the left iliac region. During laparotomy, the position of the cecum relative to the midline of the abdomen and its size were assessed (Fig. 1). Measurements were made with a centimeter tape.

Based on the "The Laboratory Rat" guidelines by Krinke [6] and the obtained own topographical data, an original intraperitoneal injection protocol was developed (certificate for rationalization proposal No. 5, dated 28.02.2020). The manipulation was performed on animals of subgroup No. 3 (n=20).

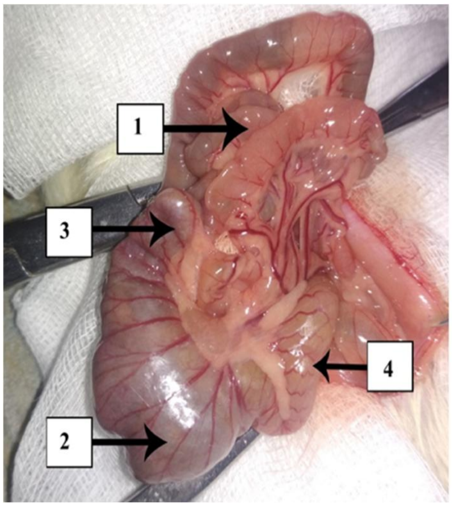


Fig.1 Macroscopic evaluation of the small and large intestine of a male Wistar rat (1- loops of the small intestine; 2- body of the cecum; 3- apex of the cecum, 4- ascending colon)

Monitoring of 8 animals that were not subject to an acute experiment was carried out after 48 and 96 hours. 5 animals of subgroup No. 3, which underwent a repeated laparotomy, were excluded from the further study, taking into account the previous mobilization of the intestinal segments, the collection of material and the severity of the sebaceous process of the peritoneal cavity.

Statistical processing of the data was performed in the analysis program STATISTICA v.10.0 (StatSoft, USA). The data are presented taking into account the mean value of indices (M) and the value of the mean standard error (m) in the form of M±m.

Results of the study and their discussion. With a wide median laparotomy, hollow parts of the digestive system (stomach, small and large intestine), liver, and bladder were visualized in the wound. The cecum in the form of a bag-like expansion measuring: l=29.3±2.9 mm, h=16.3±2.6 mm, was located in the lower quadrants of the abdominal cavity. Macroscopic data of perforation or bleeding from its wall during the examination were not found in both subgroups.

The position of the cecum was classified under three categories: right-sided (>2/3 of the length (l) in the right iliac region), median (the midline crosses relatively equal segments of the intestine), left-sided (>2/3 l in the left iliac region), as presented in Table 1.

Table 1

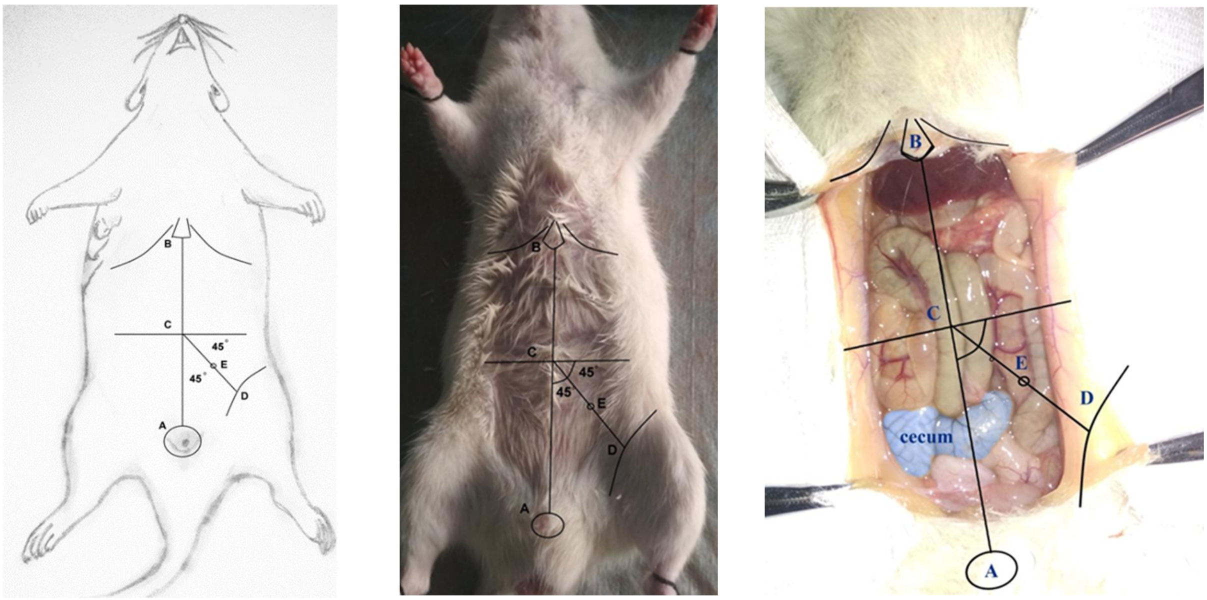
Assessment of the cecum's position in the abdominal cavity (n=43)

Group of animals / Position of the cecum	No.1 (n=14)	No.2 (n=14)	No.3 (n=15)	Total quantity (n=43)	%
>2/3 l in the right iliac region **, n**	7	6	6	19	44
Median position**, n**	4	4	5	13	30
>2/3 l in the left iliac region**, n**	3	4	4	11	26

When assessing the position of the cecum in the three subgroups, in 44 % (19/43) of the animals, the cecum was located in the right iliac region and in 26 % (11/43) in the left, respectively.

Taking into account the visible landmarks of the rat's body, namely: the opening of the preputial sac, the cartilaginous part of the xiphoid process, the inguinal folds and the predominant localization of the cecum, in 74 % (32/43) – right-sided and medial, a method of calculating the site of intraperitoneal injection was proposed, which took into account these anatomical features and was carried out as follows.

If a single injection of the medicinal drug is needed in a sedated (Metamisole sodium 50 % – 0.1 ml/kg + Chlorpromazine hydrochloride 2.5 % – 0.1 ml/kg IV) laboratory animal, the median line from the external opening of the urethra was drawn with a colored marker (part of the preputial sac) (A) to the xiphoid process (B). Through the point that divides it in half (C), a perpendicular was drawn along the left half of the abdomen's front wall. A bisector formed at an angle of 90° to the inguinal fold was lowered into the left inguinal area (D). The injection site was defined as a point lying on ½ of the line formed by the bisector of the angle (E). (Fig. 2)



A B C

Fig. 2 Schematic image (A) and topographical anatomy of the abdominal wall of a rat (B) with a defined site of intraperitoneal injection (the animal is fixed), view during laparotomy (C).

There is no need to use a marker in a previously fixed and sedated animal, which is subject to surgical intervention, which significantly reduces the intervention time. Before the injection, the left iliac region was treated three times with a 10 % solution of Betadine or 96.0 % ethyl alcohol. For intraperitoneal injection, an insulin syringe of 1 ml U-40, 29 G (0.33×12.7) was used, the animal was placed in the Trendelenburg position (40°), to move the organs of the abdominal cavity to the upper quadrants and prevent their damage. The needle was inserted at an angle of 45° to the surface of the anterior abdominal wall until the feeling of its "falling in" (4–6 mm).

When performing an injection to animals, before repeated laparotomy, a characteristic "sink" was not felt. When the syringe piston was pulled back, no blood flowed into its reservoir. Entry into the peritoneal cavity was recorded when the needle penetrated 4–6 mm. In our opinion, the cause of this phenomenon is a coupling process in the peritoneal cavity after the primary laparotomy, repeated intraperitoneal injections.

In 7 out of 8 animals subject to monitoring, signs of purulent-inflammatory process in the abdominal cavity were not detected macro- and microscopically.

One animal from subgroup No. 3 (1/15; 6%) developed soft tissue infiltration when an anesthetic (ketamine) was administered. The animal was re-injected using a new syringe. After 48 hours, induration of the injection site was noted. After 96 hours, signs of ulceration of a skin area measuring 0.5×0.5 cm appeared. Among the main complications of intraperitoneal administration of drugs are considered: iatrogenic damage to the organs of the abdominal cavity, mainly the liver, cecum, which leads to infection or bleeding, and the penetration of the drug into the surface layers of the abdominal wall, with the development of necrosis in the affected area [8]. Variability in the cecum position and the risk of liver and stomach damage lead to differences in the site for safe intraperitoneal injection.

Since the cecum is one of the largest hollow organs in the rat abdomen, injection into the lower quadrants of the abdomen is potentially dangerous [5, 10]. Factors affecting its location include the intrauterine rotation of the intestines, the size of the peritoneal cavity, a tendency to obesity, gender, and repeated surgical interventions [7].

In our study, in 74 % of cases (32/43), the cecum was located on the right and along the midline, while the median position of the cecum was noted in 30 % (13/43), which cannot be leveled at all. This position is taken into account in the calculation of the injection point, which permits the procedure to be carried out safely even in the case of a median position of the cecum.

A fundamental work on the intrauterine rotation of the intestines in rat embryos ("The embryology of gut rotation", Kluth D., 2003) indicates that the position of the intestines depends on two embryological processes: 1) the growth rate of the duodenal loop at the early stages of development and 2) the sequence of the return of the intestines into the abdominal cavity. According to his assessment, the caecum in all embryos was located on the right, closer to the ventral surface of the anterior abdominal wall. This hypothesis indicates that the cecum occupies a position in the peritoneal cavity, where there is free space ("free space"), which is rather a result of passive movement than growth activity. Passive displacement and return of the midgut into the abdominal cavity is also described on the basis of microcomputed tomography data by Ginzel M. et al. [4].

The definition of the injection point was described in the UBC Animal Care Guidelines, which was defined as the center of an equilateral triangle formed by the midline of the abdomen, a line drawn through the second row of nipples and the genital area on the right side [8]. Given the data obtained suggesting the feasibility of left-sided injection and insufficient visualization of the nipples in male rats, we prefer fixed landmarks: the xiphoid cartilage, the preputial sac, and the inguinal fold.

In addition, we believe that the position of the animal plays an important role in the preparation for the injection, preference is given to the Trendelenburg position, which promotes displacement of the organs in the rostral direction, as well as the size of the injection needle, the diameter and length of which can determine significant perforation [1]. In the recommendations – UBC Animal Care Guidelines, it is suggested to use 23–25 G needles for rats [8]. However, based on the results of effective general analgesia and sufficient throughput for a drug volume $\leq$ 2.0 mL, we recommend using a 29 G (0.33 x 12.7) needle for injecting anesthetics and solutions that do not contain dispersed particles, which contributes to less traumatization of soft tissues and pain sensations. Fecal autosuspension, filtrates of excrements penetrate freely into the peritoneal cavity using a 23 G needle.

Conclusion

Performing an intraperitoneal injection requires taking into account the topographical and anatomical features of the cecum position in sexually mature male Wistar rats.

The proposed technical solution has significant advantages: it justifies the place and point of intraperitoneal injection and reduces the risk of iatrogenic damage to abdominal organs.

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