SOME ASPECTS OF ENISAMIUM IODIDE NASAL SPRAY SAFETY: PRE-CLINICAL STUDY RESULTS

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METHA. Обґрунтування безпеки використання нового назального спрею з Енісаміумом Йодидом за результатами дослідження гострого місцевоподразнювальної дії досліджуваного препарату одноразовому введенні у кон'юнктивальні мішок та носовий хід кролів.

MATERIALI TAT METODY. Енісаміум Йодид 10 мг/мл (назальний спрей) був об’єктом тестування. Референтним препаратом був 0,9 % розчин натрію хлориду. Для індукуції експерименту використовували кролів породи Фландр (Фламандський Гігант) (2 групи, по 9 кролів у кожній групі). Об’єкти дослідження вводили одноразово в очні кон’юнктивальні мішок (0,01 мл) та носові ходи (0,1 мл) шляхом інстиляції. Очи ніг зведеніми проводили в різні часові точки спостереження (через 1, 24, 48 та 72 години після введення препаратів). Назальна ендоскопія застосовувалася для контролю стану слизової оболонки порожнини носа на всіх стадіях дослідження (за 15 хвилин до, 1 годину та 24 години після інстиляції лікарських засобів) під загальною анестезією. Шкала оцінки використовувалася для об’єктивності результатів.

РЕЗУЛЬТАТИ. Загальний бал становив 0 балів у всіх групах у всі періоди часу спостереження за відповідною шкалою та шкалою оцінки слизової оболонки порожнини носа кролів за результатами ендоскопії носа. Це відповідало стану здорового ока та здорової слизової оболонки носа.

ВИСНОВКИ. Енісаміум Йодид 10 мг/мл (назальний спрей) при одноразовому введенні в кон’юнктивальні мішок та носовий хід кролів не виявляє місцевоподразнювальної дії на кон’юнктиву ока та слизову порожнину носа експериментальних тварин. Назальна ендоскопія може бути використана як інформативний візуальний метод у доклінічних дослідженнях.

КЛЮЧОВІ СЛОВА: гостра місцевоподразнювальна дія, Енісаміум Йодид (назальний спрей), назальна ендоскопія, одноразове введення, релевантна шкала оцінки.

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The aim of the study to substantiate the safety using of the new nasal spray with anti-inflammatory action, which contains EI at a concentration of 10 mg/mL administered intranasally via study results of acute local drug-induced irritant action of the single-dose of the test object to eyes and nasal cavity mucosa. We developed the study design, which is shown in Fig. 1.

![Fig. 1. Design of the pre-clinical study «Acute local drug-induced irritant action of the test objects in the single-dose to eyes and nasal cavity mucosa»:](image)

- the studied animal group where 0.9 % saline were instilled;
- – the studied animal group where Enisamium Iodide 10 mg/mL (nasal spray) were instilled.

### 2. Material and Methods

EI nasal spray (JSC Farmak, Ukraine) with an active substance concentration of 10 mg/mL was the test object. This concentration has been chosen considering the previous stage results of the of EI (nasal spray) pre-clinical studies [8–11]. The reference drug was 0.9 % saline.

Eighteen Flemish Giant rabbits of both sexes aged around 90–110 days weighing 2.5–3 kg were used to induce the experiment. Study of local drug-induced irritant action was conducted according to the methodological recommendations «Pre-clinical study of the local drug-induced irritant action» [12]. The baseline data related to all study groups are illustrated in Table 1.

The eye examination we performed 1 hour before instillation of the study objects for initial control of eye condition, as described in the methodological recommendations [12]. Observations were made through 1, 24, 48 and 72 h after drug administration (instillation of the study objects in single-dose into the conjunctival sac of the eyes). Local drug-induced irritant action on the eye mucous membranes was studied on the relevant scale of evaluation of the indicators: the state of the tissues around the eyes, including eyelids, the condition of the conjunctiva, blinking membrane, cornea and iris in each animal.

Nasal endoscopy was used for control of nasal cavity mucosa in all stages of study (15 minutes before instillation of the study objects into the nasal passage, 1-hour and 24 hours after drug instillation with digitally capturing). The procedure was performed under general anesthesia with Ketamine/Xylocaine (35/5 mg/kg, intramuscularly) [15] using Karl Storz rhinosopes (Germany): the one was diameter 4 mm, length 150 mm, 30° angle and the other was diameter 2.7 mm, length 110 mm, 0° angle. The pictures were digitally captured using the rhinoscope and then examined to reveal the pathological changes. The condition of the nasal cavity mucosa was evaluated according to the following nasal endoscopy parameters: swelling and hyperemia of the middle turbinate, amount and character of discharge into the nasal passage (Table 2).

### Table 1

| Animal groups | Study object        | Single-dose       | Route of administration               |
|---------------|---------------------|-------------------|---------------------------------------|
| I group       |                     |                   |                                       |
| (n=9)         | I a – intact control| 0.9 % saline      | 0.01 mL                               |
|               | group (n=9)         |                   | Instillation into the conjunctival sac of the right eye |
|               | I b\(^b\) (n=6)     | Enisamium Iodide 10 mg/mL (nasal spray) | 0.01 mL |
|               | I c\(^c\) (n=3)     |                   | Instillation into the conjunctival sac of the left eye |
| II group      | II a                | 0.9 % saline      | 0.1 mL                                |
| (n=9)         |                     |                   | Instillation into the right nasal passage |
|               | II b                | Enisamium Iodide 10 mg/mL (nasal spray) | 0.1 mL |
|               |                     |                   | Instillation into the left nasal passage |

Note: ‘n’ – total number of the experimental animals; ‘n’ – number of the experimental animals in each groups; I b\(^b\) – the animals, which eyes were not washed after a single instillation of the test object; I c\(^c\) – the animals, which 20 seconds after a single instillation of the test object, the eyes were washed with water at room temperature for 1 minute.
Statistical analysis of the results was performed using Kruskal-Wallis one-way analysis of variance and Mann-Whitney U test for a posteriori pairwise comparisons [16, 17]. Utilized computer software included IBM SPSS Statistics v. 22 (IBM Corp., USA) and MS Excel 2016 (Microsoft Corp., USA). The level of statistical significance was considered as p<0.05.

3. Results

In the observation of animals after instillation of the studied objects, no changes in the animal behavior were observed, which could serve as signs of pain or discomfort. Signs of short-term blepharospasm (from 10 to 90 seconds) were observed in groups I a, I b and I c, which is most likely associated with the procedure of instilling the studied objects in the eye than with the properties of these drugs. These phenomena stopped on their own and did not require any additional treatment. In general, during of the eye examination at all-time points of observation, there were no signs of irritation. The eyes of all animals in I b and I c studied groups remained unchanged compared to the control eye (I a group) (Fig. 2). The cornea remained transparent without signs of turbidity; its surface was smooth and shiny. The iris of the left eye of the studied animals looked intact; a positive reaction to light was quick and full. None of the animals had erythema, edema and secretions (Fig. 2).

The total score on the relevant scale [12] was 0 points in all groups at all-time points, which corresponds to the condition of a healthy eye.

In studying the acute local drug-induced irritant action of the test object single-dose on the nasal mucosa, the application of the studied drugs did not attract significant changes in the general condition and behavior of animals. The combination of drugs given by materials and methods (general anesthesia with Ketamine/Xylazine) led to the formation of sufficient depth and duration of drug sleep; however, all animals were in satisfactory condition, alert and active after its completion. General anesthesia created satisfactory conditions for nasal endoscopy conducting before the instillation of the studied objects and 1-hour after this instillation (this was the first and the second episode of general anesthesia). Repeated general anesthesia (24 hours after the studied object instillation) also did not cause any negative changes either in the general condition or in the behavior of the animals. Nasal endoscopy allowed an intravital objective assessment of the condition of the nasal mucosa of experimental animals, significantly increased the possibility of an objective assessment, and allowed the material to be digitally captured for further analysis that is more thorough. In addition, a significant fact in favor of intravital nasal endoscopy is that this technique allows you to more closely follow modern bioethical standards for conducting experiments on animals, causing them the least possible harm.

Table 2

| Middle turbinate swelling | Middle turbinate hyperemia | The presence of discharge into the nasal passage | Discharge character | Points |
|---------------------------|---------------------------|-----------------------------------------------|------------------|-------|
| No changes                | No changes                | Within normal limits                          | Mucous discharge | 0     |
| Mild swelling             | Mild hyperemia            | Presence of a small amount of discharge / Slight dryness | Watery discharge | 1     |
| Moderate swelling         | Moderate hyperemia        | Presence of moderate amount of discharge / Moderate dryness | Watery-hemorrhagic discharge | 2     |
| Significant swelling      | Diffuse hyperemia         | Presence of abundant discharge / Significant dryness | Hemorrhagic or purulent discharge | 3     |

Fig. 2. Left eye condition of I b and I c groups in a different time point of observation (Enisamium Iodide 10 mg/mL (nasal spray) instillation into the conjunctival sac): a, b – in 1 hour; c, d – after 24 hours; e, f – after 48 hours; g, h – after 72 hours

Therefore, the results of nasal endoscopy of the right (intact) nasal passage in II a group (before, 1-hour and 24 hours after the studied object instillation) were as follows (Fig. 3):
the nasal mucosa was pale pink;
- on the surface of the mucosa was a small amount of transparent mucous discharge;
- sometimes a vascular pattern was visualized, consisting of unexpanded and unconvoluted vessels that were not overfilled with blood;
- no areas of hyperemia or swelling were observed.

The nasal endoscopy of the II a group showed:
- the nasal mucosa was pale pink with a small amount of transparent mucous discharge;
- the vascular pattern was unchanged;
- without signs of hyperemia or swelling.

The nasal endoscopy of the II b group showed:
- the nasal mucosa was pale pink with a small amount of transparent mucous discharge;
- the vascular pattern was unchanged;
- sometimes a vascular pattern was visualized, consisting of unexpanded and unconvoluted vessels that were not overfilled with blood;
- no areas of hyperemia or swelling were observed.

The same rhinoscopic pattern was observed with nasal endoscopy of the left nasal passage in animals of group II b (Fig. 4):
- the nasal mucosa was pale pink with a small amount of transparent mucous discharge;
- the vascular pattern was unchanged;
- without signs of hyperemia or swelling.

The total score was 0 points in both groups (II a and II b) at all-time points according to the scale of the assessment of rabbit nasal cavity mucosa by nasal endoscopy results (Table 2). Thus, it is possible to assume that the test object Enisamium Iodide 10 mg/mL (nasal spray) at the single-dose administration did not show local drug-induced irritant action on the rabbit nasal mucosa in our experiment.

However, it is worth noting that in the nasal rhinoscopy evaluation, we observed a single line hemorrhages of up to 3 mm in length on nasal mucosa into right and left nasal passages in some animals (four animals in II a group and three animals in II b group) (Fig. 3, 4). Probably, these phenomena are associated with the procedure of nasal endoscopy, during which it is impossible to completely avoid minor trauma to the nasal mucosa, but this does not affect the condition of the mucosa and animals in general.

Therefore, we can argue that nasal endoscopy is one of the most effective and safe methods of visual assessment in experimental rhinology and could be used in preclinical studies of new drugs.

4. Discussion

After statistical analysis of the total score on the relevant scale (Table 2) of the comparative study results obtained we have not observed acute local drug-induced irritant action of the test objects in the single-dose installation into the rabbit nasal passages. The total score was 0 points in both groups (II a and II b) at all-time points according to the scale of the assessment of rabbit nasal cavity mucosa by nasal endoscopy results (Table 2). Thus, it is possible to assume that the test object Enisamium Iodide 10 mg/mL (nasal spray) at the single-dose administration did not show local drug-induced irritant action on the rabbit nasal mucosa in our experiment.

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At all stages of research, we will plan to use nasal endoscopy, as the most informative visual method in experimental rhinology.

6. Conclusions

Test object Enisamium Iodide 10 mg/mL (nasal spray) has no local irritant effect on rabbit eye conjunctiva.

Test object Enisamium Iodide 10 mg/mL (nasal spray) has no local irritant effect on the rabbit nasal cavity mucosa.

It is possible to assume that Enisamium Iodide 10 mg/mL (nasal spray) does not have local irritant properties.
Enisamium Iodide 10 mg/mL (nasal spray) is a perspective object for further pre-clinical studies and clinical trials aiming to substantiate its safety and effectiveness for ARS treatment as well as its implementation to the clinical practice.

Nasal endoscopy could be used in preclinical studies of new drugs as an informative visual method for assessment in experimental rhinology.

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Conflicts of interest
The authors declare no conflicts of interest.

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1. Вступ

Різноманітними дослідженнями накопичено багато інформації по визначну роль перинатального періоду у формуванні особистості та життєвого шляху людини. Загалом, можна констатувати, що найбільш важливим значення для розвитку Его має