https://doi.org/10.17816/uroved35132

POSSIBILITIES OF PROTEOLYTIC ENZYMES IN THE TREATMENT OF SOME FORMS OF MALE INFERTILITY

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For citation: Neymark AI, Neymark BA, Nozdrachev NA, et al. Possibilities of proteolytic enzymes in the treatment of some forms of male infertility. *Urology reports (St. Petersburg)*. 2020;10(3):217-221. https://doi.org/10.17816/uroved35132

Received: 10.07.2020 Revised: 10.08.2020 Accepted: 23.09.2020

In the study 46 men with impaired fertility and the presence of viscosipathy were included. The patients' age ranged from 22 to 45 years, the average duration of infertility in marriage was 3.5 ± 2.4 years. All patients with Longidaza drugs according to the following scheme were treated. First, ten intramuscular injections of Longidaza® lyophilisate were performed to prepare a solution of 3000 IU once every five days, then Longidaza® suppositories 3000 IU rectally once every three days were prescribed, for a total of 15 suppositories. The total duration of the course of treatment is 90 days. A decrease in sperm viscosity from 2.1 ± 0.05 cm to 0.6 ± 0.07 cm ($p < 0.05$), an increase in total sperm motility (from 32.6 ± 2.9 to 61 ± 3.1%, $p < 0.05$) and the number of sperm with progressive movement (from 22 ± 1.1 to 36.7 ± 2.9 %, $p < 0.05$) after the treatment were revealed. Also, in the course of treatment, the restoration of the antioxidant protection of the ejaculate was noted, as evidenced by an increase in the activity of glutathione peroxidase and superoxide dismutase and a decrease in the content of malon dialdehyde. During the first year after the end of treatment, conception in marriage was registered in 21 (45.7%) patients. The results of the study indicate that a violation of the lipid peroxidation system can lead to both an increase in sperm viscosity and a disruption in the activity of acrosomal enzymes, as a result of which it becomes difficult for spermatozoa to penetrate the ovum. Longidaza is able to increase the hyaluronidase activity of the sperm acrosome, thereby improving the fertilizing ability of the ejaculate.

Keywords: infertility in marriage; viscosipathy; antioxidant protection; Longidaza.

ВОЗМОЖНОСТИ ПРОТЕОЛИТИЧЕСКИХ ФЕРМЕНТОВ В ЛЕЧЕНИИ НЕКОТОРЫХ ФОРМ МУЖСКОГО БЕСПЛОДИЯ

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Для цитирования: Неймарк А.И., Неймарк Б.А., Ноздрачев Н.А., и др. Возможности протеолитических ферментов в лечении некоторых форм мужского бесплодия // Урологические ведомости. – 2020. – Т. 10. – № 3. – С. 217–221. https://doi.org/10.17816/uroved35132

Поступила: 10.07.2020 Одобрена: 10.08.2020 Принята к печати: 23.09.2020

Под наблюдением находились 46 мужчин с нарушением фертильности и наличием вискозипатии. Возраст пациентов составлял от 22 до 45 лет, средняя продолжительность бесплодия в браке — 3,5 ± 2,4 года. Всем больным проводили лечение препаратами Лонгидазы по следующей схеме. Сначала выполняли 10 внутримышечных инъекций препаратом Лонгидаза® лиофилизат для приготовления раствора 3000 МЕ один раз каждые 5 дней, после чего назначали Лонгидаза® суппозитории 3000 МЕ ректально один раз каждые 3 дня, всего на курс 15 суппозиториев. Общая продолжительность курса лечения составляла 90 дней. Проведенное лечение способствовало снижению вязкости спермы с 2,1 ± 0,05 до 0,6 ± 0,07 см ($p < 0,05$) и сопровождалось увеличением общей подвижности сперматозоидов (с 32,6 ± 2,9 до 61 ± 3,1 %, $p < 0,05$) и количества сперматозоидов с прогрессивным движением (с 22 ± 1,1 до 36,7 ± 2,9 %, $p < 0,05$). Также в процессе лечения отмечено восстановление антиоксидантной защиты эякулята, о чем свидетельствовало повышение активности глутатионпероксидазы и супероксиддисмутазы и снижение содержания малонового диальдегида. В течение первого года после окончания лечения зачатие в браке зарегистрировано у 21 (45,7 %) пациента.

Результаты проведенного исследования свидетельствуют, что нарушение системы перекисного окисления липидов может приводить как к повышению вязкости спермы, так и к нарушению активности акросомальных ферментов, в результате чего сперматозоидам становится сложно проникнуть в яйцеклетку. Препарат Лонгидаза способен повышать гиалуронидазную активность акросомы сперматозоидов, тем самым улучшая оплодотворяющую способность эякулята.

Ключевые слова: бесплодие в браке; вискозипатия; антиоксидатная защита; Лонгидаза.

INTRODUCTION

The prevalence of infertility in married couples ranges from 8% to 29%, and 10% to 25% couples fail to conceive more than one child [1, 2]. The contribution of the male factor to infertility in marriage is estimated at 40%–50%, with a tendency toward a decrease in spermatogenic function in the population as a whole [3]. Recently, researchers have paid considerable attention to the causes of infertility in marriage, which, until recently, have not been considered important factors in male infertility. These involve viscosipathy and hyperproduction of reactive oxygen species (ROS). Viscosipathy (increased sperm viscosity) is relatively common; its significance lies in the complexity of the translational sperm movement and thus, the ovum fertilization. Most commonly, viscosipathy is caused by inflammatory diseases of the male reproductive system and is influenced by a sedentary lifestyle and work, improper diet, etc. In more than one-third of patients, the cause of viscosipathy cannot be identified. The use of proteolytic enzymes is recommended for the treatment of men with this type of viscosipathy.

In recent years, overproduction of ROS has also been considered a factor that reduces male fertility [4–7]. Small amounts of ROS are required for normal regulation of sperm function, their hyperactivation, and acrosomal response [8, 9]. However, excessive ROS production can be accompanied by damage to the membrane and DNA of spermatozoa and a decrease in their motility leading to apoptosis of spermatozoa and, ultimately, impaired fertility [10–14].

Increased sperm viscosity and impaired sperm penetration into the ovum are some of the least understood causes of male infertility. To fertilize an ovum, the spermatozoid must dissolve the membrane produced by the granulosa cells and then penetrate the thick membrane of the ovum. For this purpose, the proteolytic enzymes found therein are released from the acrosome. Therefore, this study aimed to analyze the impact of drugs based on proteolytic enzymes on the fertilizing capacity of the ejaculate in patients with idiopathic viscosipathy. Considering the essential function of hyaluronidase in the penetration of sperm into the ovum, Longidaza® was used as it provides a wide range of biological activity, including enzymatic proteolytic (hyaluronidase) activity of prolonged action.

MATERIALS AND METHODS

The study included 46 men with viscosipathy aged 22–45 years who had no conception in marriage. Further, the average duration of infertility in marriage was 3.5 ± 2.4 years. All subjects signed informed consent to participate in the study. Sexual partners were also examined by a gynecologist, and no diseases leading to impaired fertility were identified.

Patients were examined under the generally accepted scheme in compliance with the recommendations of the World Health Organization [2] and underwent semen analysis, endocrine testing, prostate gland and scrotum ultrasound examination, as well as blood screening for antisperm antibodies (MAR test). Additionally, the state of the antioxidant protection of the ejaculate was assessed by determining the level of malondialdehyde and the activity of antioxidant enzymes, namely, glutathione peroxidase and superoxide dismutase. These studies were conducted both before and after treatment.

All patients were treated with Longidaza® under the following scheme: first, 10 intramuscular injections of Longidaza® lyophilisate were administered to prepare 3000 IU solution once every 5 days, after which 15 3000 IU Longidaza® suppositories were administered rectally once every 3 days. The overall period of treatment was 90 days.

Generally accepted statistical approaches were used to interpret the findings of the analysis. The numerical characteristics of the clinical and laboratory parameters, namely, the mean and its standard error, median and interquartile ranges, and relative frequencies, were calculated. The difference between the mean values of the indicators was considered significant at $p < 0.05$.

RESULTS

No pathological changes were identified in the hormonal status of patients before and after treatment. The levels of total blood testosterone, luteinizing hormone, follicle-stimulating hormone, prolactin, estradiol, and thyroid-stimulating hormone were within the reference levels in all patients before and after treatment. Additionally, no major abnormalities in spermogram parameters, such as the spermatozoa morphology, their concentration, ejaculate volume, and number of leukocytes and spermatogenesis cells in the ejaculate, were noted in the course of treatment.

All the patients included in the study had an increased sperm viscosity (2.1 ± 0.05 cm) prior to treatment. Furthermore, the sperm viscosity decreased significantly, more than three times (0.6 ± 0.07 cm) in the spermogram assessment 90 days after the start of therapy. The difference in values before and after treatment was significant ($p < 0.05$). Additionally, the decline in sperm viscosity was accompanied by a significant increase in overall sperm motility (from 32.6 ± 2.9 to 61 ± 3.1%; $p < 0.05$) and increase in the amount of spermatozoa with progressive movement (from 22 ± 1.1 to 36.7 ± 2.9%, $p < 0.05$). Simultaneously, the level of sperm DNA fragmentation was within the normal range in patients of before and after treatment groups (12 ± 2.4 and 11 ± 2.3%, respectively).

In the course of this study, an important conclusion was drawn that, in addition to increased sperm viscosity, men with impaired fertility had impaired antioxidant protection of the ejaculate. This was evidenced by an increase in malondialdehyde (4.1 ± 0.3 μmol/L) and a decrease in antioxidant enzyme activity, namely, glutathione peroxidase (284.7 ± 21 U/L) and superoxide dismutase (136 ± 14 U/ml). This antioxidant system disorder possibly caused the low fertilizing capacity of the ejaculate in the patients followed up. Examination after the 90-day course of treatment with Longidaza® preparations showed significant changes in the enzyme activity of the ejaculate antioxidant system; as the activity of glutathione peroxidase increased to 482 ± 16.3 U/L, the activity of superoxide dismutase increased to 192 ± 18.3 U/ml and the level of malondialdehyde in the ejaculate decreased to 2.6 ± 0.5 μmol/L. This change in the values of all three antioxidant protection indicators relative to the pre-treatment level is statistically significant ($p < 0.05$).

Treatment effectiveness was proven by the fact that conception in marriage was registered in 21 (45.7%) patients in year 1 after completion.

The results of this study indicate that an impairment of the lipid peroxidation system can lead to both an increase in sperm viscosity and a disruption in the activity of acrosomal enzymes, resulting in the difficulty of the sperm to penetrate the ovum. The drug Longidaza® is capable of increasing the hyaluronidase activity of the sperm acrosome, thereby enhancing the capacity of the ejaculate to fertilize.

CONCLUSIONS

1. Increased viscosity of the ejaculate is accompanied by a decrease in the ability of the sperm to penetrate into the ovum, which may be attributed to impaired acrosomal action.

2. ROS are essential for the normal regulation of spermatogenesis, but their excessive production can lead to impaired ejaculate fertility, possibly due to a negative effect on acrosomal response.

3. The drug Longidaza® has a beneficial influence on the sperm viscosity and lipid peroxidation system, thereby improving the fertilizing ability of the ejaculate and increasing the frequency of conception in patients with idiopathic viscosipathy.

REFERENCES

1. Божедомов В.А., Теодорович О.В. Эпидемиология и причины аутоиммунного мужского бесплодия // Урология. – 2005. – № 1. – С. 35–44. [Bozhedomov VA, Teodorovich OV. Epidemiology and causes of autoimmune male infertility. *Urologiia.* 2005;(1):35-44. (In Russ.)]
2. Rowe PJ, Comhaire FH, Hargreave TB, Mellows HJ. WHO Manual for the Standardized Investigation, Diagnosis and Management of the Infertile Male. Cambridge University Press; 2000.
3. Тер-Аванесов Г.В. Андрологические аспекты бесплодного брака. Практическое руководство. – М., 2000. – 48 с. [Ter-Avanesov GV. Andrologicheskie aspekty besplodnogo braka. Prakticheskoe rukovodstvo. Moscow; 2000. 48 p. (In Russ.)]
4. Калинина С.Н., Кореньков Д.Г., Фесенко В.Н. Лечение сперматологических нарушений и оксидативного стресса после перенесенных репродуктивно значимых заболеваний, вызванных инфекциями, передающимися половым путем // Урологические ведомости. – 2018. – Т. 8. – № 4. – С. 5–15. [Kalinina SN, Koren'kov DG, Fesenko VN. Treatment of spermatologic disorders and oxidative stress after reproductively significant diseases caused by sexually transmitted infection. *Urologicheskie vedomosti.* 2018;8(4):5-15. (In Russ.)]. https://doi.org/10.17816/uroved845-15.
5. Agarwal A, Said TM. Oxidative stress, DNA damage and apoptosis in male infertility: a clinical approach. *BJU Int*. 2005;95(4): 503-507. https://doi.org/10.1111/j.1464-410X.2005.05328.x.
6. Tremellen K, Miari G, Froiland D, Thompson J. A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment. *Aust N Z J Obstet Gynaecol*. 2007;47(3):216-221. https://doi.org/10.1111/j.1479-828X.2007.00723.x.
7. Гамидов С.И., Иремашвили В.В., Тхагопсоева П.А. Терапия нарушения фертильности у мужчин: перспективные результаты европейских исследований // Эффективная фармакотерапия. – 2009. – № 26. – С. 26–31. [Gamidov SI, Iremashvili VV, Tkhagopsoeva PA. Terapiya narusheniya fertil'nosti u muzhchin: perspektivnye rezul'taty evropeyskikh issledovaniy. *Effektivnaya farmakoterapiya*. 2009;(26):26-31. (In Russ.)]
8. Aitken RJ, Gordon E, Harkiss D, et al. Relative impact of oxidative stress on the functional competence and genomic integrity of human spermatozoa. *Biol Reprod*. 1998;59(5):1037-1046. https://doi.org/10.1095/biolreprod59.5.1037.
9. De Lamirande E, Gagnon C. The dark and bright sides of reactive oxygen species on sperm function. In: The male gamete: from basic science to clinical application. Ed. by C. Gagnon. Vienna: Cache River Press; 1999. P. 455-467.
10. Алоян К.А., Матвеев А.В., Морев В.В., Корнеев И.А. Физиологические механизмы обеспечения подвижности сперматозоидов // Урологические ведомости. – 2013. – Т. 3. – № 4. – С. 14–19. [Aloyan KA, Matveev AV, Morev VV, Korneev IA. Physiology of sperm motility. *Urologicheskie vedomosti.* 2013;3(4):14-19. (In Russ.)]. https://doi.org/10.17816/uroved3414-19.
11. Громенко Д.С. Применение наукоемких технологий для оценки фертильности мужчин // Вестник новых медицинских технологий. – 2008. – Т. 15. – № 4. – С. 118–120. [Gromenko DS. The use of scientific technologies for estimation of man's reproductive function. *Journal of new medical technologies*. 2008;15(4):118-120. (In Russ.)]
12. Kao SH, Chao HT, Chen HW, et al. Increase of oxidative stress in human sperm with lower motility. *Fertil Steril*. 2008;89(5): 1183-1190. https://doi.org/10.1016/j.fertnstert.2007.05.029.
13. Kemal Duru N, Morshedi M, Oehninger S. Effects of hydrogen peroxide on DNA and plasma membrane integrity of human spermatozoa. *Fertil Steril*. 2000;74(6):1200-1207. https://doi.org/10.1016/s0015-0282(00)01591-0.
14. Loft S, Kold-Jensen T, Hjollund NH, et al. Oxidative DNA damage in human sperm influences time to pregnancy. *Hum Reprod*. 2003;18(6):1265-1272. https://doi.org/10.1093/humrep/deg202.

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