UDK 615.9:616.36-099:576.2.24:577.124-127

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Effects of two antituberculosis agents compositions on DNA fragmentation in Wistar albino male rats

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Key words: DNA fragmentation, testes, epididymises, rat

The pharmacotherapy of tuberculosis by various combinations of antibiotics is always associated with the possibility of serious undesirable consequences development. Moreover, even knowing the long-term consequences of each individual medicines use does not allow us to foresee with confidence their effects in combination with other antituberculosis agents. On the other hand, the severity of this pathology and the duration of treatment require the most possible optimizing of the used means choice.

The aim of investigation was a comparative primary assessment of two antituberculosis medicines combinations (ATM) possible long-term consequences for male reproductive function. For this purpose, we estimated the DNA fragmentation level in rat testis and epididymises as a marker of apoptosis [1].

Material and methods. Wistar albino male rats, body weight (b.w.) of 150 g to 170 g, were used in the study. They were kept under a controlled temperature (from 22 °C to 24 °C), relative humidity of 40 % to 70 %, lighting (12 h light-dark cycle), and on a standard pellet feed diet («Phoenix» Ltd., Ukraine). The study was performed in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Institutional Animal Care and Use Committee.

Ethambutol (EMB), isoniazid (INH), pyrazinamide (PZA), rifampicin (RMP) were supplied by the SIC «Borzhagovsky Chemical-Pharmaceutical Plant» CJSC, Ukraine. ATM suspended in 1 % starch gel were given by gavage in DOTS (directly observed treatment, short-course) regimen at maximal doses used in clinic [2] EMB – 155,0 mg/kg B.W./day, RMP – 74,4 mg/kg B.W./day, INH – 62,0 mg/kg B.W./day, PZA – 217,0 mg/kg B.W./day – for 60 days (duration of spermatogenesis process and time of germ cell maturation in epididymis [3]). Streptomycin (STR) dose was 14 mg/kg B.W./day. STR (supplied by the SIC «Borzhagovsky Chemical-Pharmaceutical Plant» CJSC, Ukraine) was dissolved in novocain solution produced by SIC «Darnitsa», series: 10111 and injected intramuscularly also for 60 days. The coefficient for conversion of human doses to animal equivalent doses based on body surface area was taken into account [4].

Animals were divided into 3 groups (8 rats in each):

Group 1: control – administration of 1 % starch gel;

Group 2: co-administration administration of INH, RMP, PZA and EMB for 60 days (combination 1);

Group 3: co-administration of INH, RMP, PZA and STR for 60 days (combination 2).

At the terminal phase of the experiments, rats were euthanised by decapitation under diethyl ether anesthesia. The ether concentration was 80 ml per liter of container volume; exposure time was approximately 5 min. Removed testes and epididymises were stored in liquid nitrogen till further investigation.

To determine the degree of DNA fragmentation the specimens (frozen in liquid nitrogen) were homogenized and genomic DNA was obtained [5].

Tissue was homogenized and digested in digestion buffer (100 mM NaCl; 10 mM Tris-HCl; 25 mM EDTA, pH 8; and 0,5 % SDS) and freshly added 0,1 mg/mL

proteinase K (Sigma-Aldrich, Ink., USA) (1:1,2 mg/ml) with shaking at 50 °C for 15 h. RNA was degraded by incubation of the samples with 1–100 mg/mL thermostable RNase H for 1,5 h at 37 °C. DNA was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) and centrifuged for 10 min at 1700 x g. The DNA was precipitated by adding 0,5 vol 7,5 M ammonium acetate and 2 vol 100 % ethanol to the aqueous layer; samples were separated by centrifugation at 1700 x g for 5 min, rinsed with 70 % ethanol, and air-dried. The pellet was dissolved in TBE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8); and then were fractionated through 2 % agarose gels (50–60 V; 3,5 h). After electrophoresis gels were stained with ethidium bromide and visualized under a UV transilluminator (BIORAD, USA). Electrophoresis data analysis was carried out with Quantity One Software (USA).

Results. The effects of two ATM combinations on DNA fragmentation in rat testes and epididymises were investigated.

Figure 1 demonstrates that in the testes of control animals was present only a slight fragmentation of DNA (2 fractions in the area of 20–100 b.p.).

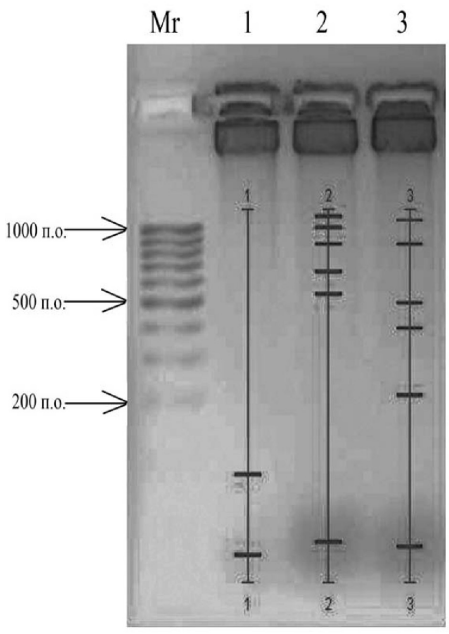

Figure 1. DNA fragmentation in rat testes (Mr – DNA marker; 1– control; 2 – combination 1; 3 – combination 2). Analysis conducted using the software Quantity One

The administration of combination 1 enhanced testes DNA fragmentation and resulted in the formation of 6 major fractions of DNA fragments. The first fraction was represented by relatively high-molecular fragments – 1200 b.p., the second – 850 b.p., the third – 650 b.p. and the fourth – 550 b.p. The fifth fraction consists of low molecular weight fragments of DNA (40–30) b.p. According to peak intensity data, the first and fifth fractions of DNA were the largest (Figure 1).

The combination 2 also caused formation of 6 main fractions of DNA fragments: the first – with lengths exceeding 1000 b.p., the second – 850 b.p., the third – 500 b.p., the fourth – 400 b.p., the fifth – 200 b.p. and the sixth – 40–30 b.p. The largest of them was a fraction containing DNA strands in the range 40–30 b.p. According to the peak intensity data it was 1,5 times greater than others.

It has been established that the relative content of DNA fragments in the testes of rats receiving two different combinations of ATMs increased by 3–4 times compared with control (Table).

A slightly different picture was observed in the study of rat epididymises DNA fragmentation (Fig. 2).

In case of only starch gel administration (control), there was a slight fragmentation of DNA to 3 main fractions (the first – 250 b.p., the second – 150 b.p. and the third – 20 b.p.). According to the peak intensity data the second and third fractions of DNA were the largest.

Combination 1 administration enhanced fragmentation in epididymises and resulted in the formation of 10 major DNA fractions. The first fraction was represented by relatively high-molecular fragments of 900 b.p., the second – 800 b.p., the third – 750 b.p., the fourth – 550 b.p., the fifth – 500 b.p., the sixth – 400 b.p., the seventh – 350 b.p., the eighth – 250 b.p. The ninth and tenth fractions were represented by low molecular weight fragments of DNA – 30 and 40 b.p. According to peak intensity data, the first, sixth and eighth fractions of DNA were the largest (Figure 2).

Table

Relative content of DNA fragments of different molecular weights in the testes and epididymises of rats receiving two combinations of ATM

Group	% of DNA fragmentation from total DNA content	
	testes	epididymises
Control	6,51	6,53
Combination 1	22,22	46,81
Combination 2	28,29	36,54

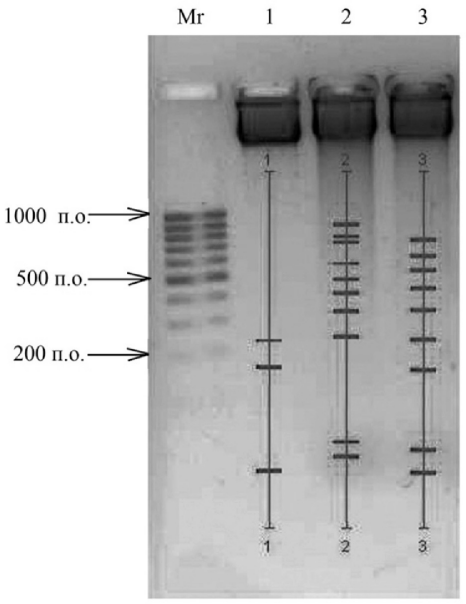

Figure 2. DNA fragmentation in rat epididymises (Mr – DNA marker; 1 – control; 2 – combination 1; 3 – combination 2). Analysis conducted using the software Quantity One

When combination 2 was administered to experimental animals, in their epididymises 9 major fractions of DNA fragments were detected in the range from 20 b.p. up to 800 b.p. According to peak intensity data these fractions had approximately equal peaks intensity.

Relative contents of DNA fragments in rats epididymises with administration of ATM combinations 1 and 2 were greater than that in control by 7 and 5, 6 times, respectively (Table).

Discussion. DNA is an important molecular target for antituberculosis agents toxic effects [6], which induced endonucleases for its lethally splitting. Among this such compounds could inhibit processes of DNA repair by nuclear DNA-polymerases. Level and character of DNA fragmentation are markers of apoptotic processes in organism [1].

In our experiments, obviously, in testes and epididymises, the toxic effects of the ATMs combinations were realized irrespective of STR or EMB presence. Although certain features of their effects were discovered. The combination with EMB more strongly stimulated the processes of DNA fragmentation in epididymises, whereas the use of a combination with STR – in testes. Certain differences in the fragmentation of DNA between epididymises and testes may be associated with different nucleases sets in various tissues.

The results obtained are in good correspondence with our previous data on changes of DNA fragmentation processes in hepatocytes and testes of rats with administration of EMB, INH, PZA and RMP alone or in combination [7–10] and with results of other authors, which established antituberculosis agents negative effects on liver DNA methylation state [11], and inhibition of normal DNA synthesis in spermatogonia of male rabbits [12]. Dysregulation of physiological germ cells apoptosis, which could cause male infertility [13], may be a result of external disturbances such as exposure to certain chemotherapeutic agents [14]. Differences in DNA-fragmentation processes between control and ATM combinations groups could be caused by changes in effectiveness DNA-fragmentation processes [1]. The comparatively high level of DNA fragmentation in testes of ATM-treated male rats is an evidence of germ cells apoptotic death intensification [15].

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Key words: DNA fragmentation, testes, epididymises, rat

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Вплив двох композицій протитуберкульозних засобів на фрагментацію ДНК у щурів-самців лінії Вістар

Фармакотерапія туберкульозу різними комбінаціями антибіотиків завжди пов'язана з можливістю розвитку серйозних небажаних наслідків. Більше того, навіть знаючи про довготривалі наслідки використання кожного лікарського засобу, ми не можемо з упевненістю передбачити їхні наслідки в

поєднанні з іншими протитуберкульозними агентами. З іншого боку, тяжкість цієї патології та тривалість лікування вимагають максимально можливої оптимізації вибору використовуваних засобів.

Мета дослідження – порівняльна первинна оцінка можливих довготермінових наслідків щодо чоловічої репродуктивної функції двох комбінацій протитуберкульозних лікарських засобів. З цією метою оцінено рівні фрагментації ДНК у сім'яниках та епідидимісах щурів як маркери апоптозу. Тварин поділяли на 3 групи (по 8 щурів у кожній): група 1 – контроль – введення 1 % крохмалю; група 2 – одночасне застосування ізоніазиду, рифампіцину, піразинаміду та етамбутолу протягом 60 днів (комбінація 1); група 3 – одночасне застосування ізоніазиду, рифампіцину, піразинаміду та стрептоміцину протягом 60 днів (комбінація 2). У наших експериментах, очевидно, у сім'яниках та епідидимісах токсичні ефекти комбінацій протитуберкульозних засобів реалізовувалися незалежно від наявності стрептоміцину або етамбутолу, хоча були виявлені певні особливості їхніх наслідків. Комбінація з етамбутолом сильніше стимулювала процеси фрагментації ДНК в епідидимісах, тоді як застосування комбінації з стрептоміцином – у сім'яниках. Деякі відмінності в рівнях фрагментації ДНК між епідидимісами та сім'яниками можуть бути пов'язаними з різними наборами нуклеаз у даних тканинах. Порівняно високий рівень фрагментації ДНК у сім'яниках самців, що пройшли лікування протитуберкульозними засобами, є свідченням інтенсифікації в них апоптотичної загибелі статевих клітин.

Ключові слова: фрагментація ДНК, сім'яники, епідидиміси, щури

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Влияние двух композиций противотуберкулезных средств на фрагментацию ДНК у крыс-самцов линии Вистар

Фармакотерапия туберкулеза различными комбинациями антибиотиков всегда связана с возможностью развития серьезных нежелательных последствий. Более того, даже зная о долговременных последствиях использования каждого лекарственного средства, мы не можем с уверенностью предсказать их последствия в сочетании с другими противотуберкулезными агентами. С другой стороны, тяжесть этой патологии и продолжительность лечения требуют максимально возможной оптимизации выбора используемых средств.

Цель исследования – сравнительная первичная оценка возможных долгосрочных последствий относительно мужской репродуктивной функции двух комбинаций противотуберкулезных лекарственных средств. С этой целью мы оценили уровни фрагментации ДНК в семенниках и епидидимисах крыс в качестве маркеров апоптоза. Животных разделяли на 3 группы (по 8 крыс в каждой): группа 1 – контроль – введение 1 % крахмала; группа 2 – одновременное применение изониазида, рифампицина, пиразинамида и этамбутола в течение 60 дней (комбинация 1); группа 3 – одновременное применение изониазида, рифампицина, пиразинамида и стрептомицина в течение 60 дней (комбинация 2). В наших экспериментах в семенниках и епидидимисах токсические эффекты комбинаций противотуберкулезных средств, очевидно, реализовывались независимо от наличия стрептомицина или этамбутола, хотя были выявлены определенные особенности последствий их введения. Комбинация с этамбутолом сильнее стимулировала процессы фрагментации ДНК в епидидимисах, тогда как применение комбинации со стрептомицином – в семенниках. Некоторые различия в уровнях фрагментации ДНК между епидидимисами и семенниками могут быть связаны с различными наборами нуклеаз в данных тканях. Сравнительно высокий уровень фрагментации ДНК в семенниках самцов, прошедших лечение противотуберкулезными средствами, является свидетельством интенсификации в них апоптической гибели половых клеток.

Ключевые слова: фрагментация ДНК, семенники, епидидимисы, крысы

Надійшла: 25 червня 2018 р.

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