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The effect of dinitrosyl iron complexes with glutathione and S-nitrosoglutathione on the development of experimental endometriosis in rats: A comparative studies

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ABSTRACT

It has been established that intraperitoneal bolus administration of S-nitrosoglutathione (GS-NO) (12.5 μmoles/kg; 10 injections in 10 days), beginning with day 4 after transplantation of two 2-mm autologous fragments of endometrial tissue onto the inner surface of the abdominal wall of rats with surgically induced (experimenta) endometriosis failed to prevent further growth of endometrioid (EMT) and additive tumors, while treatment of animals with dinitrosyl iron complexes (DNIC) with glutathione (12.5 μmoles/kg, 10 injections in 10 days) suppressed tumor growth virtually completely. The histological analysis of EMT samples of GS-NO-treated rats revealed pathological changes characteristic of control (non-treated with GS-NO or DNIC) rats with experimental endometriosis. EPR studies established the presence of the active form of ribonucleotide reductase, a specific marker for rapidly proliferating tumors, in EMT samples of both control and GS-NO-treated animals. Noteworthy, in small-size EMT and adjacent tissues of DNIC-treated rats the active form of ribonucleotide reductase and pathological changes were not found.

1. Introduction

Endometriosis is a rapidly progressing medical condition, which affects more than 10% of the female population of reproductive age (Eskenazi and Warner, 1997; Van Langendonct et al., 2008). This disease may be caused by implantation of endometrial cells and their enhanced proliferation in endometrial tissues outside the uterine cavity (most frequently, in the peritoneal cavity). In-the-state-of-the-art, the most popular treatment of this disorder consists in surgical removal of extrauterine implants and hormonal therapy. However, the use of these methods significantly increases the risk of post-surgical complications and often provokes adverse side effects. Therefore, a search for and testing of candidate drugs for effective treatment of endometriosis are currently performed on animal models of experimental endometriosis

Our previous studies demonstrated that dinitrosyl iron complexes (DNIC) with natural thiol-containing ligands (glutathione and cysteine) can effectively suppress the development of experimental endometriosis in rats at both early and more advanced stages of the neoplastic process (Burgova et al., 2012, 2014; Adamyan et al., 2013). Treatment of rats with DNIC over a period of 1–12 days, beginning with day 4 after transplantation of autologous 2-mm fragments of endometrial tissue onto the inner surface of the abdominal wall, or 1 month thereafter initiated fast (within 1 month) growth of large-size ($\leq$10 mm in diameter) benign endometrioid tumors (EMT) and suppressed their proliferative activity. It was suggested that this effect is a result of selective destruction of DNIC (both inside and outside EMT) by endogenous iron chelators in order to provide tumor tissues with iron required for their growth. The fast release of large quantities of NO from decomposing DNIC and its further oxidation by superoxide anions are accompanied by enhanced synthesis of cytotoxic peroxynitrite whose degradation products initiate the death of EMT at physiological pH.

But here another point arises: can other NO donors, e.g., S-nitrosothiols (RS-NO), also exert such selective cytotoxic effects? Earlier, it was found (Mier-Carbrera et al., 2013) that chronic (during several months) administration of S-nitrosopenicillamine

Abbreviations: B- or M-DNIC, binuclear- or mononuclear dinitrosyl iron complex; EMT, endometrioid tumors; EPR, electron paramagnetic resonance; GS-NO, S-nitrosoglutathione; HFS, hyperfine structure; MNIC-DETC, mononitrosyl iron complexes with diethyldithiocarbamate; SNAP, S-nitrosopenicillamine

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(SNAP) at significantly lower doses than the dose of DNIC used in our previous studies (Burgova et al., 2012, 2014; Adamyan et al., 2013) not only failed to suppress EMT growth in experimental mice, but even enhanced it. The assessment of the immune status of SNAP-treated rats (interleukine and interpherone tests) established a significant decrease in the rate of their synthesis in comparison with control. This finding led us to suppose that chronic administration of SNAP to rats notably worsened their immune status and, as a consequence, initiated enhanced growth of EMT.

The aim of the present study was to examine whether another widely known S-nitrosothiol, viz., S-nitrosoglutathione (GS-NO), can enhance EMT growth at early stages of experimental endometriosis induced by treatment of rats with GS-NO over a period of 1–12 days. The doses of GS-NO and DNIC with glutathione were identical and equal to 12.5 μmoles/kgwt. As in our previous studies (Burgova et al., 2014), we used the binuclear form of DNIC with glutathione (B-DNIC) (formula $[(GS)_2Fe_2(NO)_4]$), which represents a glutathione ether of Roussin's red salt (Vanin et al., 2010).

2. Materials and methods

2.1. Materials

Reduced glutathione and sodium nitrite were purchased from Sigma (St. Louis, USA). Ferrosulfate was from Fluka (Buchs, Switzerland). The freeze-dried preparation of sodium thiopental was obtained from LLC "Sintez" (Kurgan, Russia); xylazine was from Interchemie (the Netherlands). Gaseous NO was generated in the reaction of ferrosulfate with sodium nitrite in 0.1 M HCl with subsequent purification of NO by low-temperature sublimation in an evacuated glass system.

2.2. Protocol- synthesis of DNIC with glutathione

The synthesis of DNIC with glutathione was based on the ability of bivalent iron to bind to gaseous NO in aqueous solutions at neutral pH in the presence of thiols (McDonald et al., 1965). The synthesis of these complexes was carried out in a Thunberg apparatus (total volume 100 ml) at the Fe^{2+}:glutathione molar ratio of 1:2 and at gaseous NO pressure of 100 mm Hg. The ferrosulfate solution (0.5 ml) in distilled water (pH 5.5) and the glutathione solution (4.5 ml) in 15 mM HEPES buffer (pH 7.4) were loaded into the upper and lower chambers of the Thunberg apparatus, respectively. Gaseous NO was loaded into the deaerated vessel and the Fe^{2+}+glutathione solutions were mixed in the presence of NO upon continuous shaking; NO was evacuated 5 min after mixing. The solution of B-DNIC with glutathione formed thereupon had a characteristic orange color; its concentration was determined from the concentration of ferrosulfate added to the mixture and was equal to 2.5 mM (or 5 mM as calculated per one iron atom in B-DNIC). The final concentration of B-DNIC was estimated by the intensity of the characteristic absorption band at 360 nm (ε=7400 M^{-1} cm^{-1}) (Vanin et al., 2010) and was equal to 2.5 mM (5 mM as calculated per one iron atom in B-DNIC).

2.3. Protocol: synthesis of GS-NO

The synthesis of GS-NO was based on the ability of nitrous acid (HNO_2) formed as a result of nitrite protonation in acid media to S-nitrosate thiols (including reduced glutathione (GSH)) by the reaction: $HNO_2 + GSH \rightarrow GS\text{-}NO + H_2O$ (Williams, 2004).

Glutathione (5.5 mM) and sodium nitrite (5 mM) were added consecutively to a 15 mM solution of HEPES after preliminary acidification of the latter with hydrochloric acid (pH 3.0–3.5) in air, which gave the solution a characteristic reddish-pink color due to formation of GS-NO. After 1-h incubation in air, the pH of the solution was increased to neutral values to complete GS-NO synthesis. The GS-NO concentration was followed by the intensity of the optical absorption band at 334 nm and reached 5 mM.

2.4. Animals

The experiments were performed on adult female Wistar rats weighing 160–80 g. After treatment with DNIC+glutathione and S-nitrosoglutathione, the weight of animals increased to 220–250 g. The animals were provided by the "Stolbovaya" Affiliated Nursery of the Russian Academy of Medical Sciences. Throughout the observation period (45 days), the animals were housed in the vivarium of the N.M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, in compliance with the Guidelines of the Geneva Convention "International Principles for Biomedical Research Involving Animals" (Geneva, 1990).

2.5. Animal studies

2.5.1. Protocol: induction of experimental endometriosis

Experimental endometriosis in rats was simulated by using a modified surgical procedure described by Vernon and Wilson (1985). The animals were at the proestrus stage of the estrous cycle. Surgical procedures were performed in the supine position on a standard rat surgery board under thiopental anesthesia (0.06 g/kgwt) with xylazine (3 mg/kgwt) premedication and lasted 40–45 min. Tumor growth was induced by surgical transplantation of two autologous fragments (2×2 mm^2) of uterine tissue (the endometrium together with the myometrium) excised from the left uterine horn onto the anterior surface of the abdominal wall. After termination of invasive treatment, the rats were kept for 4 days under standard vivarium conditions (controlled environment, constant temperature (23 ± 2 °C, 12 h light/dark cycles). Standard dietary intake including free access to water was used to accelerate engraftment.

2.5.2. Protocol: studies on rats with experimental endometriosis

The animals were selected for the study by random sampling where body mass was used as criterion of choice. All animal studies were carried out in the autumn period (October–November). The animals were randomly divided into 3 groups: Group 1 (n=15) (control)—rats with surgically induced endometriosis without GS-NO or DNIC treatment; Groups 2 (n=9) and 3rd (n=9)—experimental animals with surgically induced endometriosis and treated with DNIC or GS-NO, respectively. All animals were kept under identical conditions and 2–4 h before the beginning of treatment were deprived of food to enable partial emptying of the bowels. Rats in the 2 group were given an intraperitoneal dose (0.5 ml) of a neutral solution of 5 mM B-DNIC with glutathione as calculated per one iron atom in B-DNIC (12.5 μmoles/kgwt). This treatment was performed daily at 11 a.m., Moscow time, beginning with day 4 after surgery, and lasted 10 days. In Group 3rd, a neutral solution of 5 mM S-nitrosoglutathione (0.5 ml) (12.5 μmoles/kgwt) was administered to rats intraperitoneally, also beginning with day 4 after surgery, (daily, for 10 days) at fixed time (11 a.m.). The animals were allotted into cages (4–5 rats in each cage) depending on the group; each cage was marked with eosin or methylene blue and after completion of the treatment course were kept under standard vivarium conditions for at least 2 weeks, after which time they were killed by decapitation under thiopental anesthesia.