INTRODUCTION

Water-soluble dinitrosyl iron complexes (DNIC) with thiol ligands (cysteine or glutathione), mono- and binuclear (respectively \{(RS–)2Fe+(NO+)2\}+ and \{(RS–)2Fe2+ (NO)4\}2+) [1] show versatile physiological effects. In animals and human volunteers, they cause prolonged hypotension [2–4] owing to their powerful vasodilatory action [5, 6]; they attenuate aggregation of platelets [7] and erythrocytes [8], at the same time enhancing the elasticity of the latter [9]; they promote skin wound healing [10]; DNIC also have a potent erectile effect, even under experimental denervation of cavernous bodies in rats [11, 12]; importantly, in prolonged administration they suppress the fibrotic transformation common in denervated cavernous tissue [11].

As shown in experiments with cultured hepatocytes and tumor cells [13], DNIC with thiol ligands prevented S-nitrosoacetyl penicillamine-induced apoptosis. These results are in accord with the data for HeLa [14]: DNIC themselves exhibited no proapoptotic effect and even suppressed spontaneous apoptosis in passaged cells. On the other hand, apoptosis was initiated upon destruction of DNIC by iron chelators; this effect was suggested to be due to rapid release of DNIC components—neutral NO molecules and nitrosonium ions (NO+) [14]. The reaction of NO with superoxide ions yielded peroxynitrite, a cytotoxic compound which can trigger apoptosis, while NO+ caused S-nitrosation of proteins on the cell surface, which could also be proapoptotic. Thus, decomposition of DNIC (e.g. in zones of lowered pH) may elicit cytotoxicity, which can be seen at least as suppressed cell/tissue proliferation. To check this possibility, in the present work we have studied the action of DNIC on the development of experimental endometriosis in rats.

Endometriosis is defined as the presence of endometrial glandular tissue outside of the uterus; proliferation of endometrial tissue within the myometrium (formerly known as endometriosis interna) is currently termed adenomyosis; both are among the major causes of female sterility. The multiplicity of factors that supposedly determine the origin and progression of endometriosis substantially complicates its treatment. In this connection, a search for new therapeutics against this disease is quite a topical task.

EXPERIMENTAL

All experiments were conducted in conformity with the International Guiding Principles for Biomedical Research Involving Animals (Geneva, 1990).

Endometriosis was induced in Wistar female rats (160–180 g, Stolbovaya nursery) in proestrus by a modified autotransplantation model [15], whereby a fragment of endometrium with myometrium (2 × 2 mm) from the left uterine horn was grafted to the inner surface of the anterior abdominal wall (endometrium facing the peritoneal cavity). Surgery was performed under thiopental anesthesia (0.06 g/kg body wt) within 40–45 min. Then the animals were kept in a vivarium under a 12/12 light regime and constant temperature (23 ± 2°C) on a standard diet with...
free access to water; after surgery, sterile chip bedding was replaced daily. Four weeks were allowed for engraftment and development of endometrioid foci. Then the rats were randomly divided into two groups. The test animals received intraperitoneal injections of 0.5 mL 5 mM DNIC-Cys daily for 10 days. The controls received an equal volume of saline. After this course, the animals were kept under the same conditions for one week more, and then sacrificed under thiopental narcosis. Thus, the total duration of the experiment for animals was 45 days.

After opening the abdominal cavity, the endometriomas were measured (length, width, height), photographed, separated and subjected to histological examination. The tissue was placed in 20% formalin; after standard processing and paraffin embedding, the specimens were stained with hematoxylin and eosin. The cyst volume was determined with the formula for ellipsoids, length × width × height × π/6 (mm³) [16]. Statistical processing involved the Student’s parametric test.

Binuclear DNIC-Cys was synthesized as earlier [17] in a Thunberg tube, mixing ferrous sulfate (Fluka) and L-cysteine solutions (final 5 mM each) under gaseous NO at 100 mmHg. The resulting solution was frozen and stored in liquid nitrogen, to be thawed immediately before use.

For EPR measurements, endometrioma specimens were taken from two rats in 30 min after the last DNIC injection and one day later, as well as from control rats, and immediately frozen in liquid nitrogen. Spectra were recorded at 77 and 293 K with a Bruker ESC-106 X-range radiospectrometer at an HF modulation amplitude of 0.2 mT and microwave power of 5 mW.

RESULTS AND DISCUSSION

A total of 21 animals were operated in the experiment. In the post-surgery period, we observed characteristic behavior and postures indicative of engraftment and endometrioma development with the attending pain syndrome [18]. A histological check 14 days after grafting confirmed that an active endometrioid focus developed on the abdominal wall. The endometrium was represented by an active stromal component and small glands with a narrow round lumen lined by cubic or cylindrical epithelium (i.e., resting or proliferating). Weak inflammatory infiltration could be seen in the endometrial stroma. At the graft boundary, the abdominal wall contained large loci of leukocytic infiltration including macrophages, which was indicative of chronic inflammation, but this did not spread onto the graft and no destruction loci were revealed therein.

At autopsy in the end of the experiment (day 45), endometrioid cysts were found in all rats, control and DNIC-treated. If an animal had more than one cyst, their volume was summed. The mean volume of endometriomas in the control group was 32.4 ± 9.7 mm³ (n = 5). In the treated group (one week after a 10-day course of DNIC-Cys, n = 8), it was 17.5 ± 10.3 mm³ (54% of control). The difference was statistically significant.

A conventional way to express the effect of a drug against tumor-like growth is the ratio of the mean size (area or volume) of the pathological formations in the control and in the treated group (i.e., reduction factor). In our case, this ratio is 1.85. Thereby DNIC-Cys compares favorably with most of contemporary pharmaceuticals tested against endometriosis in the rat model. It is superior to pentoxifylline [19], cetrorelix, leuprolide [20], and rosiglitazone [21], for which the size ratios were within 1.6 despite a much longer treatment (up to eight weeks); greater reduction factors have been reported only for leflunomide [22] and raloxifene [23]: 4.1 and 2.3, but these were also obtained after longer courses (four and two weeks respectively) while the control endometriomas grew to much larger sizes.

Samples of endometriomas from control and DNIC-treated rats were subjected to EPR spectroscopy. Specimens taken 30 min after the final injection of DNIC-Cys and examined at 77 K exhibited an intense anisotropic signal with $g_\perp = 2.04$, $g_\parallel = 2.014$ $g_{av} = 2.03$ (Fig. 1a), coinciding with the spectrum of...