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Diacerein inhibits Estradiol-benzoate induced cervical hyperkeratosis in female rats

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ABSTRACT

Cervical hyperkeratosis is a common gynecological lesion and usually caused by inflammation or trauma. We investigated the effect of Diacerein on Estradiol benzoate-induced cervical hyperkeratosis. Diacerein (50 mg/kg/day) was given orally to rats for 4 weeks in the presence or absence of cervical hyperkeratosis induced by intramuscular injection of Estradiol benzoate (60 μg/100 g) 3 times per week for 4 weeks. We measured the serum levels of total cholesterol, uterine weights, uterine tissue malondialdehyde, total nitrites, superoxide dismutase activity, caspase-3, interleukin-1b immunoexpression and histopathology. Our results showed that Estradiol benzoate succeeded to induce cervical hyperkeratosis which was detected by typical histopathological changes. In addition; there was significant reduction in superoxide dismutase levels and caspase-3 immunoexpression but significant increase in serum total cholesterol, malondialdehyde, total nitrites and interleukin-1b immunoexpression. Diacerein could improve all measured parameters to normal levels. It markedly prevented cervical hyperkeratosis through its anti-inflammatory (IL-1b receptor inhibitor), antioxidant and anti-apoptotic effects.

1. Introduction

Cervical hyperkeratosis (CHK) is the presence of a thickened keratin layer on the surface of stratified squamous epithelium.CHK is usually related to inflammation, trauma or infection, and it is commonly seen in women who use diaphragm [1,2].

The traditional screening test for cervical cancer is Pap test. It has been proved to be one of the most successful tests in decreasing malignant rates. Human papilloma virus (HPV) has been linked to almost all cervical squamous cell carcinoma preceded by high grade squamous intraepithelial lesion (HSIL). As screening tools, Pap and HPV tests are subjected to false negative and false positive results. False negativity is usually attributed to screening and sampling errors, also the presence of an intrinsic pathophysiology in the cervix as hyperkeratosis may be another cause of false negativeresults [3].In Pap smear, CHK is characterized by the presence of anucleated squamous cells either singly or in sheets [2].

According to the 2001 BETHESDA System Types of cervical squamous cell abnormalities include: 1-Atypical squamous cell of undetermined significance (ASC-US) and cannot exclude HSIL (ASC-H), 2-Low grade squamous intraepithelial lesion (LSIL) encompassing HPV/mild dysplasia/CIN1, 3-High grade squamous intraepithelial lesion (HSIL) encompassing moderate and severe dysplasia, 4-Squamous cell carcinoma [2].

Uterine cervix is highly responsive to estrogen [4,5]. During the menstrual cycle, cervical epithelial cells proliferate and differentiate with increasing estrogen levels, resulting in hyperplastic epithelium without pathological changes. Administration of estrogen in high doses or for long duration contributes to the induction of cervical lesions as CHK [6]. Animal models exposed to Estradiol benzoate (EB) showed evidence suggestive of cervical carcinoma with stromal invasion [7].

Excessive estrogen administration inhibits apoptotic pathways including caspase-3 pathway leading to enhanced DNA replication and repair errors. This in turn causes somatic mutations and subsequent malignancy [8].

Exposure to unopposed estrogen induces an inflammatory response in the uterus [9]. The proinflammatory cytokines and inflammatory mediators promote neoplastic transformation. This can also increase estrogen production, which may facilitate carcinogenesis by disrupting the estrogen-progestogen balance [8]. Inflammation induces rapid cell division with an increase in the concentration of free radicals causing DNA damage [8,10].

Interleukin-1 (IL-1) is an important cytokine; it stimulates lymphocytes proliferation, activates macrophage and initiates an

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inflammatory cytokine cascade.Interleukin-1b (IL-1b) is the predominant form of IL-1 produced by many cells [11]. Cytokines are involved in uterine hyperplastic changes and carcinoma [12].

Diacerein (DIA) is an anti-inflammatory (IL-1b receptor inhibitor), analgesic and antipyretic drug. It was produced for treatment of osteoarthritis [13]. It inhibits IL-1 production by monocytes and it has potent antioxidant activity [14].

It was approved that DIA has marvelous protective effects in different models. DIA provides a novel strategy with better efficacy for breast cancer therapy through its anti-proliferative and apoptotic effects [15,16]. It decreased the viability of human chondrosarcoma cells and induces G2/M cell cycle arrest by CDK1/cyclin B1 down-regulation [17]. It could be used in the treatment of psoriasis [18] and doxorubicin induced nephrotoxicity [13].

EB induces CHK via increasing IL-1b production and free radicals formation. We aimed to study the effect of IL-1b receptor antagonist, powerful antioxidant and anti-apoptotic agent (DIA) on EB induced CHK.

2. Materials and methods

2.1. Chemicals

DIA powder was from Eva Pharma Company, Egypt. EB 5 mg ampoule was from Misr Company, Egypt. Polyclonal caspase-3 and IL-1b anti-bodies were purchased from Thermo Fisher Scientific Inc./Lab Vision (Fermont, CA, USA).

2.2. Animals and experimental design

Adult female Wistar rats weighing between 250 and 300 g were from the animal house, Giza, Egypt. Animals were kept in standard housing conditions in cages, 3 rats/cage. They were left to acclimatize for one week. Rats were supplied with laboratory chow and tap water. This work was conducted in the Pharmacology Departement, Faculty of Medicine, Minia University, Egypt. The animal experimental protocol was approved by the faculty board in accordance with European (EU) directive 2010/63/EU.

Induction of CHK was performed with administration of EB (60 μg/100 g) by intramuscular injection (i.m) in quadriceps muscle 3 times per week for 4 weeks always at the same time.The most effective dose of DIA is 50 mg/kg/day orally as found in different articles [13,19]

The dose of DIA for each rat was diluted in 1 ml 1% carboxymethylcellulose and it was given at the same time orally.

DIA was given concomitantly with the administration of EB to evaluate its prophylactic effect to prevent the induction of CHK.

Rats were randomly divided into 4 groups; each group (n = 6):

Group I (control) received vehicle (1% carboxymethylcellulose) [13] and i.m injection of olive oil 3 times per week for 4 weeks [20].

Group II (diacerein group) was given DIA (50 mg/kg/day orally) [13] and i.m injection of olive oil 3 times per week for 4 weeks [20].

Group III (cervical hyperkeratosis induced group) was given vehicle (1% carboxymethylcellulose) [13] and i.m EB (60 μg/100 g) 3 times per week for 4 weeks [21].

Group IV (diacerein + cervical hyperkeratosis induced group) was given DIA (50 mg/kg/day orally) [13] plus i.m EB (60 μg/100 g) 3 times/week for 4 weeks [21].

At the end of 4 weeks; the animals were sacrificed and venous blood samples were collected from the jugular vein, centrifuged at 5000 rpm for 15 min (JanetzkiT30 centrifuge, Germany).

2.3. Evaluation of serum total cholesterol

The total cholesterol was determined after enzymatic hydrolysis and oxidation. The quinoneimine was formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. The produced color intensity was measured colorimetrically at 500 nm by Beckman DU-64 UV/VIS, USA spectrophotometer. Results were expressed as mg/dl [22].

Table 1
Effect of DIA on serum total cholesterol in EB induced CHK.

Group	Cholesterol (mg/dl)
Control	57.83 ± 2.242
DIA	60.83 ± 1.922
CHK	101.2 ± 2.509[a]
CHK + DIA	66.67 ± 3.783[b]

Values are representation of 4–6 observations in each group as means ± S.E.M. DIA is diacerein group; CHK is cervical hyperkeratosis induced group. Results are considered significantly different when $P < 0.05$.

[a] Significant difference compared to control.

[b] Significant difference compared to Estradiol benzoate group.

Table 2
Effect of DIA on uterine weights and SOD in EB induced CHK.

Group	Uterine weights/g	SOD(unit/g tissue)
Control	0.960 ± 0.02	825.5 ± 30.69
DIA	1.000 ± 0.05	783.5 ± 30.01
CHK	9.567 ± 0.3[a]	627.8 ± 22.08[a]
CHK + DIA	3.267 ± 0.2[a,b]	810.3 ± 15.22[b]

Values are representation of 4–6 observations in each group as means ± S.E.M. DIA is diacerein group, CHK is cervical hyperkeratosis induced group. Results are considered significantly different when $P < 0.05$.

[a] Significant difference compared to control.

[b] Significant difference compared to Estradiol benzoate group.

Table 3
Effect of DIA on MDA and NO_X levels in EB induced CHK.

Group	MDA(μmol/g tissue)	NO_X (μmol/g tissue)
Control	2.635 ± 0.22	63.50 ± 1.7
DIA	2.422 ± 0.19	65.58 ± 3.7
CHK	13.78 ± 0.84[a]	128.7 ± 3.9[a]
CHK + DIA	3.193 ± 0.22[b]	76.83 ± 4.9[b]

Values are representation of 4–6 observations in each group as means ± S.E.M. DIA is diacerein group, CHK is cervical hyperkeratosis induced group. Results are considered significantly different when $P < 0.05$.

[a] Significant difference compared to control.

[b] Significant difference compared to Estradiol benzoate group.

2.4. Preparation of uterine homogenate

After sacrifice, uterus was excised and weighed on Mettler Toledo scale, Swizer Land. Each uterus was divided into 2 parts. One part was stored at −80 °C for homogenate, and the other part was fixed in 10% formaldehyde,embedded in paraffin and used for histopathology and immunohistochemistry.

For preparing of uterine homogenate for biochemical analysis (MDA, SOD, NOx), uterus was homogenized (Glas-Col homogenizer, USA), and a 20% w/v homogenate was prepared in ice-cold phosphate buffer (0.01 M, pH 7.4). The homogenate was centrifuged at 3000 rpm for 20 min, and the supernatant was kept at −80 °C till used.

2.5. Evaluation of uterine superoxide dismutase (SOD) levels

Evaluation of uterine antioxidant defense mechanisms was detected by assessment of uterine tissue SOD enzyme levels.

The measurement of uterine SOD levels were based on that the oxidation of pyrogallol was inhibited by SOD. One unit of SOD is defined as the amount of enzyme that inhibits the oxidation of pyrogallol