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Original Research Article

Evaluation of antioxidant activity of ormeloxifene: in vitro study

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ABSTRACT

Background: Ormeloxifene (Centchroman) is a Selective Estrogen Receptor Modulator (SERM) which acts as estrogen antagonist and having anti progestogenic activity also. It is being used in the management of dysfunctional uterine bleeding and as nonhormonal oral contraceptive. It is also being investigated for the indications such as osteoporosis, breast and endometrial carcinoma. In this study, we have evaluated the Antioxidant potential of drug by using DPPH and NO synthase Assay. It was found that ormeloxifene has significant antioxidant activity which could be cause for its use in various gynaecological and other conditions.
Methods: In this study, we have demonstrated in vitro antioxidant activity of ormeloxifene. DPPH and NO synthase assay tests were done for different concentrations of ormeloxifene.
Results: In our study, it showed that the free radical scavenging activity of ormeloxifene was less in lower concentration and increased in the higher concentration in DPPH assay. The free radical scavenging activity of drug ormeloxifene was 22% at 100µg/ml and 27% for the concentrations of 1000µg/ml in DPPH assay. No scavenging activity was 3% at 100µg/ml and 11% at 1000µg/ml.
Conclusions: The invitro antioxidant analysis of ormeloxifene, was proved to be a potent antioxidant.

Keywords: DPPH (1,1-diphenyl-2-picrylhydrazy) assay, Nitric oxide synthase assay, Ormeloxifene, Selective estrogen receptor modulator

INTRODUCTION

Ormeloxifene is a 3rd generation selective estrogen receptor (ER) modulator. It is nonsteroidal estrogen antagonist which has high affinity to the ERs and shows estrogenic activity in some tissues such as vagina, bone, cardiovascular, and central nervous system and have antiestrogenic effect in breast and uterus.[1] The chemical name of ormeloxifene is trans-7-methoxy-2, 2-dimethyl-3-phenyl-4 (4-(2-pyrrolidinoethoxy) phenyl (-chromanhydrochloride).[2] The drug competitively binds with cytosol receptors and not only blocks them but also cause prolonged depletion, so that its action lasts longer even after withdrawal of drug. It is well absorbed from the GI tract, attains a peak level in 4 h, and its terminal half-life is 170 h. It has little affinity to plasma proteins.

It is mainly used for the treatment of dysfunctional uterine bleeding associated with anovular cycles in premenopausal women. It is best known as a nonhormonal, nonsteroidal oral contraceptive taken once a week. It is marketed as centchroman since 1990s.[3] It is developed at CDRI India and introduced in the National Family Welfare Programme to be distributed as an oral contraceptive under brand name 'SAHELI'. The contraceptive action is probably due to utero-embryonic asynchrony and failure of implantation.[4] The advantages over hormonal oral contraceptives are, being it producing lesser incidence of vomiting, vertigo, weight gain, hypertension and

breakthrough flow. It does not causes any thrombotic episodes, adverse effects on lipid profile and also no risk of cancer. It does not have androgenic, antiandrogenic or progestational properties. It does not affect the secretions of pituitary, thyroid or adrenal hormones in its contraceptive doses. If pregnancy occurs, the treatment should be discontinued and pregnancy should follow the natural course as there is no risk of teratogenesis with centchroman.[5] Prominent side effects are nausea, headache, prolongation of menstrual cycles. Jaundice or hepatic dysfunction, polycystic ovarian disease, cervical hyperplasia, tuberculosis, renal disease or hypersensitive with centchroman are contraindications for its use. Further, it is being investigated for the treatment of osteoporosis, breast and endometrial carcinoma. There is a considerable evidence for its Anti-inflammatory activity and used for various gynaecological conditions. In this present study, we have evaluated the Antioxidant potential of ormeloxifene by using DPPH and NO synthase Assay.

METHODS

Free radical scavenging activity of ormeloxifene was determined by DPPH Assay method as per Yohozowa et al.[6] The nitric oxide radical scavenging activity of ormeloxifene was done by using method of Alderson et al method 2001.[7]

DPPH scavenging activity

The free radical scavenging activity of Ormeloxifene was determined by using DPPH radical scavenging activity by using the method of Yohozowa et al.[6] This assay is based on the principal of reduction of absorbance of ethanol solution of DPPH by free radical scavenger. Reagents required for this assay were DPPH and Ethanol. The reaction mixture containing 1ml of DPPH solution (200µM in ethanol) with different concentration of the test drug (25, 50, 100, 200, 400, 800, 1000µg/ml) was shaken and incubated in dark for 20min at room temperature. The resultant absorbance was recorded at 517nm by using Spectrophotometer. Ormeloxifene free radical scavenging activity (The percentage inhibition) was calculated using the formula.

$$\text{Percentage inhibition} = \frac{(\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{Sample})}) \times 100}{\text{Abs}_{(\text{control})}}$$

Nitric oxide scavenging activity

The nitric oxide radical scavenging activity was done using the method of Alderson et al.[7] It is based on the principal of inhibition of nitric oxide radical (which is generated from sodium nitro pruside in phosphate buffed saline with addition of griess reagent). Griess reagent contains 1% sulphilamide, 2% phosphoric acid and 0.1% naphthyl ethulene diamine dihydrochloride in 100ml of distilled water. 3ml of reaction mixture containing sodium nitroprusside (10mM in phosphate buffer saline) and various concentrations (25, 50, 100, 200, 400, 800µg/ml) of the test drug were incubated at 37^0C for 4 hours. To the incubation solution, 0.5ml of Griess reagent was added and the absorbance was read at 546nm by using spectrophotometer. The free radical scavenging activity of ormeloxifene.

(The percentage inhibition) was calculated using formula

$$\text{Percentage inhibition} = \frac{(\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{Sample})}) \times 100}{\text{Abs}_{(\text{control})}}$$

RESULTS

DPPH assay was done for different concentrations of Vitamin C and ormeloxifene from 10µg/ml to 1000µg/ml. The percentage inhibition of Vitamin C 91%, 94%, 95%, 94%, 94% and for drug ormeloxifene was 23%, 24%, 25%, 26%, 27% for the concentrations of 100µg /ml, 200µg/ml, 400µg/ml, 800µg/ml, 1000µg/ml respectively (Table 1). It showed dose dependent gradual increase in free radical activity.

Table 1: DPPH scavenging activity of ormeloxifene.

Concentration (µg/ml)	% Inhibition	
	Vitamin C	Ormeloxifene
10	91.02±0.02	16.04±0.04
50	94.59±0.03	18.14±0.02
100	94.96±0.11	22.36±0.01
200	95.14±0.08	23.83±0.01
400	95.51±0.06	24.65±0.01
800	94.78±0.04	25.34±0.05
1000	94.41±0.07	26.07±0.07

Values are expressed as mean±S.D of three experiments

Table 2: Nitric oxide scavenging activity of ormeloxifene.

Concentration (µg/ml)	% Inhibition	
	Vitamin C	Ormeloxifene
10	56.45±0.92	1.04±0.97
50	64.27±0.61	2.07±0.42
100	74.64±0.73	3.60±0.82
200	79.32±0.92	5.19±0.98
400	83.14±1.02	6.93±0.56
800	87.42±0.04	9.18±0.48
1000	92.12±0.52	11.63±0.81

Values are expressed as mean±S.D of three experiments

The NO synthase assay was done for different concentrations of Vitamin C and Ormeloxifene from 10 to 1000. The percentage inhibition of Vitamin C was 56%, 64%, 74%, 79%, 83%, 87%, 92% and the drug Ormeloxifene was 1%, 2%, 3%, 5%, 6%, 9% for the concentration of 10µg/ml, 20µg/ml, 50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml, 800µg/ml, 1000µg/ml. The nitric oxide radical scavenging activity of ormeloxifene was found to be much less at all concentrations from 10µg /ml to 1000µg/ml compared to standard antioxidant drug

(Ascorbic acid). The percentage inhibition was 1% at 10µg/ml and 11% at 1000µg/ml concentrations (Table 2).

DISCUSSION

Ormeloxifene (Centchroman) has been found to possess significant anti-inflammatory activity in acute as well as chronic models of inflammation.[8] It is being investigated in the treatment of osteoporosis, breast and endometrial carcinoma, where it has been implicated that the free radicals producing oxidative stress can cause oxidative damage by accumulating during the life cycle. Free radicals can produce oxidative stress. This is balanced by the body's endogenous antioxidant systems with an input from co-factors, and by the ingestion of exogenous antioxidants. If the generation of free radicals exceeds the protective effects of antioxidants, and some co-factors, this can cause oxidative damage which accumulates during the life cycle, and has been implicated in diseases such as cardiovascular disease, cancer, neurodegenerative disorders, and other chronic conditions.[9] Hence we aimed to evaluate antioxidant property of ormeloxifene.

It was done by DPPH and NO synthase assay. The DPPH assay was selected Since it is a relatively stable free radical, considered a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants.[10,11]

NO is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes. NO in excess concentration is implicated in the cytotoxic effects observed in various disorders such as AIDS, cancer, Alzheimer's and arthritis. NO in excess concentration reacts with the oxygen to generate free radicals such as nitrite and peroxynitrite anions.[12] Hence, NO synthase assay was also tested. This assay has showed that the drug does not have nitric oxide radical scavenging activity.

CONCLUSION

From our study, the free radical scavenging property as measured by DPPH method showed that percentage of inhibition increases with increase in the concentration of ormeloxifene. It is an effective antioxidant with radical scavenging potency almost similar to Vitamin C. Hence, ormeloxifene could play a role of an antioxidant in wide range of conditions. Further studies are required for demonstration for antioxidant effect in vivo.

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