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Electron emission and product analysis of estrone: progesterone interactions studied by experiments *in vitro*

MARION GERSCHPACHER¹, NIKOLA GETOFF², JOHANNES HARTMANN², HEIKE SCHITTL², IREN DANIELOVA¹, SHAOBIN YING², JOHANNES C. HUBER¹, and RUTH M. QUINT²

¹Department of Gynecologic Endocrinology and Reproductive Medicine, University Hospital of Vienna, Waehringerguertel 18–20, A-1090 Vienna, Austria

²Section Radiation Biology, Faculty of Life Science, University of Vienna, Althanstrasse 14, A-1090 Vienna, Austria

Abstract

Recent studies showed that hormones like progesterone, testosterone, etc. can eject e_{aq}^- (solvated electrons). By means of electron transfer processes via the brain, the hormones communicate with other biological systems in the organism. The present study proves that also estrone is able to emit electrons. Their yield strongly depends on the concentration of the hormone, temperature and on the absorbed energy. The metabolites resulting from this process are likewise able to generate electrons, however with much smaller yields. The formation of the estrone metabolites is studied by HPLC-analyses. *In vitro* experiments with MCF-7 cells demonstrate the distinct effect of progesterone on the carcinogenicity of estrone metabolites. Probable reaction mechanisms for explanation of the observed effects are postulated.

Keywords

Estrone; progesterone; electron emission; MCF-7; HPLC-analysis

Introduction

In recent studies, it was established that sexual hormones like 17β -estradiol (17β -E2), progesterone (PRG) [1] and testosterone (TES) [2] are able to emit electrons ('solvated electrons', e_{aq}^-) from their excited singlet state. The same capability was also observed for other hormones like the phytohormone genistein [3], 4-hydroxyestrone (4-OHE1) [4], adrenaline (ADR) [5], etc. Thereby, a part of the ejected electrons are consumed by the hormones themselves with the reaction rate constant of $k(e_{aq}^- + \text{hormone}) = 10^9$ to $10^{10} \text{ l mol}^{-1} \text{ s}^{-1}$. The main part of the e_{aq}^- , however, is transmitted to other biological systems in the organism via the brain [6]. Reference [6] presents a hypothesis for explaining the mechanisms of signals transmitted by electrons, originating from different types of hormones which renders a communication between the hormones and other biological systems. Hence, the hormones act as 'electron mediators'. A hypothesis is postulated for the specific communication mechanisms between hormones and other biological systems in the organism [6].

The transients resulting of the electron ejection process of the sexual hormones may interact with substances that are present in the actual media and form certain metabolites. Those metabolites produced by incorporation with, e.g. heterocyclic aromatic compounds (pyrene, etc.), have the ability to initiate cancer. The formation of such carcinogenic metabolites depends on nutrition, genetics, environment, etc. Several carcinogenic metabolites of sexual hormones have been studied in detail [7-10].

The naturally occurring estrogens in humans are 17β -estradiol (E2), estrone (E1), and estriol (E3). They are derived from androgenic precursors by aromatisation of the A-ring. In premenopausal women, E2 is the predominant estrogen while in postmenopausal subjects, E1 is considered as the main acting estrogen. E3, however, is synthesised in large amounts of the placenta during the pregnancy. The interaction between estrogens and various enzymes can play a role in the pathogenesis and in the development of hormone-depending breast cancer [11,12]. Moreover, E1 and E2 are able to form catechol estrogen metabolites and catechol estrogen quinons, which may interact with the DNA to form predominantly depurinating adducts. These adducts are supposed to lead to mutations that initiate cancer development [13].

The present study focused on the ability of estrone (E1) to emit e_{aq}^- in dependence of the hormone concentration, absorbed energy, and temperature as well as on the interaction between E1 and PRG regarding cancer. In this respect, HPLC-analyses and experiments *in vitro* were performed.

Methods

UV-source and actinometry

Estrone (E1; >99% purity, Sigma-Aldrich) was dissolved in an air-free mixture of 40 vol% triply-distilled water and 60 vol% p.A. ethanol in a specially designed double wall 4p-geometry irradiation apparatus and was immediately irradiated. A low pressure UV-lamp (HNS 12, OSRAM, 12 Watt) with incorporated Vycor-filter for the elimination of the 185 nm-line was used providing a monochromatic UV-light of 254 nm ($4.85 \text{ eV } h\nu^{-1}$) [14]. The intensity of the monochromatic UV-light ($I_0 = 1 \times 10^{18} \text{ } h\nu \text{ ml}^{-1} \text{ min}$) was determined by monochloroacetic acid [15]. The desired temperature during the irradiation of the solution was kept constant by means of a thermostat. The emitted e_{aq}^- of E1 were scavenged by $1 \times 10^{-2} \text{ mol l}^{-1}$ chloroethanol [16]:

Therefore:

$$Q(\text{Cl}^-) = Q(e_{aq}^-) \quad (2)$$

Product analysis

The photolytic products of E1 resulting from the electron emission process were determined by the HPLC-method (apparatus: Hewlett-Packard, model 1046/1050, with computer online) using Hepersil ODS 5 μm , $40 \times 125 \text{ mm}^2$ column, injection sample 20 μl , solvent mixture: 40% water, 10% acetonitrile and 50% methanol; flow rate: 1 ml min^{-1} .

Experiments *in vitro*

The effect of PRG on the carcinogenicity of E1 metabolites was studied by experiments *in vitro*, using MCF-7 cells as a model. The handling of the cells and the evaluation of the data

were described previously [17]. As an irradiation source for γ -ray served a 'Gammacell 220' (Nordion Ltd., Canada). The dosimetry was performed by modified Fricke-Dosimeter [18]. Microscopic pictures were taken of untreated and γ -ray treated MCF-7 cells incubated in E1, PRG and mixtures of both hormones.

Results

Electron emission

Based on previous experiments with other hormones mentioned earlier, it was expected that E1 is also able to emit e_{aq}^- when being excited in the singlet state. This process was first studied using $1 \times 10^{-5} \text{ mol l}^{-1}$ E1 at 37 and 45°C. The mean values of 4–5 different measurements are illustrated in Figure 1. The standard deviation is about 15%.

The electron emission of E1 shows two maxima at both temperatures. The second peak is attributed to the metabolites resulting from the E1 transients generated by the electron emission and from the ethanol radicals [Equation (1)]. The corresponding $Q(e_{aq}^-)$ -data are shown in Figure 1, inset I. In fact, the yield of e_{aq}^- at 45°C is much higher than that obtained at 37°C. The electron emission process is accompanied by a pH-decrease with increasing absorbed UV-dose shown in Figure 1, inset II. This observation may indicate that the OH-group of the A-ring of the estrone molecule is involved in this process.

The pH-curve has no maxima showing a continuous generation of e_{aq}^- . This effect was also previously observed in experiments with other hormones [3–5].

Experiments were also performed with $5 \times 10^{-5} \text{ mol l}^{-1}$ E1 in order to study the effect of the substrate concentration on the electron emission process. The mean values of several measurements at 37 and 45°C are presented in Figure 2. The calculated $Q(e_{aq}^-)$ data at the corresponding peaks at both temperatures are given as inset I and the observed pH-change as a function of the absorbed UV-quanta is shown in inset II, Figure 2.

As reported in previous studies [1–4], the $Q(e_{aq}^-)$ yields decrease with increasing substrate concentration as a consequence of the associate formation. As already mentioned, these unstable complexes are supposed to consume a part of the emitted electrons. The $Q(e_{aq}^-)$ -yields of several E1-concentrations are summarised in Table I.

Recent experimental findings showed that hormones may partly be oxidized during dissolving in aerated polar media leading to a decrease of the $Q(e_{aq}^-)$ -yield [19]. Compared to the samples dissolved in air-free media, the obtained $Q(e_{aq}^-)$ data of both series are given in Table I. The values are lower than those dissolved in an air-free media.

HPLC-analyses

HPLC-analyses were performed using samples of various experiments. The aim was to observe the instability of E1 during the dissolving process in aerated media as well as to study the formation of E1-metabolites originating from samples dissolved in an air-free solvent. Some of them are depicted in Figure 3.

Experiments *in vitro*

The purpose of these studies was to examine the effect of PRG on the carcinogenicity of E1 metabolites generated by free radicals produced with γ -radiation of the media. Similar free radicals are generated in the human organism. MCF-7 cells were used as a model. The results are presented as survival curves in dependence of the absorbed radiation dose, Gy (1 Gy = 100 rad = $6.24 \times 10^{15} \text{ eV g}^{-1}$ absorbed energy) under various conditions.

Figure 4 shows the survival curves N/N_0 -ratio, (N_0 = number of cell-colonies prior to treatment, N = after treatment) presented in dependence of the absorbed dose (Gy) in aerated media. In this case, oxidizing radicals ($Q_2^{\cdot-}$, OH) are the reacting species leading to the survival curves (A–D).

Studies *in vitro* were also carried out in a media saturated with N_2O used for the conversion of e_{aq}^- into OH:

Under these conditions studied survival curves are presented in Figure 5.

Moreover, experiments were also performed in buffer solutions free of oxygen. The results are shown in Figure 6. In all three cases (Figures 4-6), the course of the survival curves is rather similar, indicating the resemblance of the free radical action on the cancer cells.

Microscopic images of MCF-7 cells incubated with E1, PRG and in a mixture of both, untreated as well as treated with γ -ray (absorbed dose: 15.6 Gy) are shown in Figure 7. The strong effect of each individual hormone and of the mixture is manifested.

Discussion

Based on the Figures 1 and 2 it is obvious that the electron emission (formation of e_{aq}^-) from E1 is one of the primary processes as a consequence of the absorbed energy in the substrate. This process competes with other photophysical procedures such as fluorescence, intersystem crossing of singlet to triplet, etc. Most likely, the electrons are emitted from the π -electron structure of ring A, as previously established with phenol and related compounds [20]. The process is illustrated in Equation (4):

(4)

The observed pH-decrease (inset II in Figures 1 and 2) supports the existence of reaction (4) by the formation of H^+ anions.

On the other hand, the carbonyl group ($=CO$) on the 17th position of the E1-molecule is consuming e_{aq}^- with a rate constant, $k \sim 1 \times 10^9 \text{ l mol}^{-1} \text{ s}^{-1}$, leading to an electron-adduct (R2) [21]:

(5)

The resulting phenoxy radical type of ring (A), R1, exists in several mesomere structures. For simplicity, the phenoxy radical of phenol is taken for illustration:

(6)

Each of the above resulting mesomere forms (A–D) as well as the transients R1 and R2 can react with the actual species present in the medium to form the corresponding metabolite. As an example, the $\cdot\text{C}_2\text{H}_4\text{OH}$ radical [see Equation (1)] is the only one available reaction partner to produce a metabolite M1; e.g.:

(7)

If the metabolite (M1) becomes electronically excited in the singlet state, it is also able to emit

(8)

e_{aq}^- likewise as E1. This explains the appearance of the second maxima observed in Figures 1 and 2. The formation of metabolites is proven by the HPLC-experiments, as illustrated by the comparison of the chromatograms (C) and (D) in Figure 3. After a prolonged UV-irradiation, not only E1 but also the resulting metabolites were decomposed.

The performed experiments *in vitro* using MCF-7 cells as a model delivered interesting insights into the biological consequences resulting from the action of free radicals on E1. As a matter of fact, free radicals are permanently produced and consumed in the human organism. They play an essential role in the human organism. In the present work, γ -ray was used as a tool for the production of free radicals of the media. The cross reaction (8) outlines the radical production by water radiolysis. The yields of the produced species are given as *G*-values (*G*-value = number of species generated by 100 eV absorbed energy: 1 eV = 1.602×10^{-19} J).

As already mentioned, 4×10^{-2} mol l-ethanol was added into the aqueous media for a better dissolving of E1. Ethanol, however, is known as an efficient scavenger of OH and H species:

The reaction rate constant (k) of e_{aq}^- with ethanol is very low and can be neglected. In the presence of air, e_{aq}^- and H are converted into peroxy-radicals:

Additionally, the ethanol radicals can also, at least partly, be scavenged by O_2 :

Based on the processes shown above, the survival curves registered in the presence of air (Figure 4) are a consequence of the attack of $\text{Q}_2^{\bullet -}$, $\bullet\text{C}_2\text{H}_2\text{OH}$ and of the $\bullet\text{O}_2\text{C}_2\text{H}_4\text{OH}$ radicals. Only these species are interacting with the cells and the resulting hormone transients.

The survival curves of MCF-7 cells as a function of the absorbed dose (Gy) in the presence of E1 and PRG represent the overall effect of the mentioned process. Nevertheless, the antitumour effect of E1 (curve B), of PRG (curve C) and of the mixture of both are strongly pronounced. In particular, the antitumour action of the hormone mixture (curve D) shows a synergism at relative low radiation dose of 5–6 Gy, which is of practical importance since the permitted applicable radiation dose for cancer patients is 5 Gy.

The tendency of the course of the survival curves of the same systems, but in a media saturated with N_2O (Figure 5) is very similar to the curves shown in Figure 4. In the present case, however, the e_{aq}^- are converted into OH radicals [Equation (3)] that are immediately scavenged by ethanol producing $\bullet\text{C}_2\text{H}_4\text{OH}$ radicals [Equation (9)]. Hence, oxygen [Equation (11)] and ethanol [Equation (10)] compete for the H-atoms [Equations (10) and (11)] whereby reaction (11) is predominant. As a final result of all involved processes, the $\bullet\text{O}_2\text{C}_2\text{H}_4\text{OH}$ and the radicals are again interacting with E1 and PRG and their transients are involved in the generation of the observed survival curves shown in Figure 5. The higher similarity of the course of these survival curves compared to those shown in Figure 4, confirm the antitumour property of E1 and PRG transients.

In Figure 6, survival curves of MCF-7 cells incubated under the same conditions, but in air-free media, are shown. In this case, the primary radicals e_{aq}^- , H and OH [Equation (8)] are involved in the reactions with the compounds in the media. Because of the relative high concentration of ethanol, reactions (9) and (10) are predominant and the resulting $\bullet\text{C}_2\text{H}_4\text{OH}$ radicals react with the cells as well as with E1 and PRG. The solvated electrons (e_{aq}^-), however, interact simultaneously with E1 and PRG, leading to the corresponding electron

adducts [see Equation (5)]. As a consequence of all involved processes, the observed survival curves show a similarity to those survival curves presented in Figures 4 and 5.

The antitumour effect of E1 and PRG transients and especially of their mixture is also visualised by some microscopic images of MCF-7 cells in aerated media containing ethanol (Figure 7). Further data are given in the legend of the figure.

Regarding the clinical relevance, it can be stated that hormones have multibiological functions: they have the ability to emit electrons, as well as to consume a part of them. The main fraction of the emitted electrons, however, is transferred to other biological systems via the brain, enabling a communication between hormones and the biological systems. Within these processes, PRG might play an important role in generating a radical cation ($\text{PRG}^{\bullet+}$) as a consequence of the electron emission. Afterwards, $\text{PRG}^{\bullet+}$ can be bound to other hormone radicals resulting in metabolites with decreased carcinogenic metabolites.

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Figure 1. Electron emission of $1 \times 10^{-5} \text{ mol l}^{-1}$ estrone in an air-free mixture of 40% water and 60% ethanol as a function of the absorbed UV-quanta at 37 and 45°C. Inset I: $Q(e_{aq}^-)$ values at the corresponding maxima. Inset II: pH-change of the media in dependence of the absorbed quanta. Inset III: structure formula of estrone.

Figure 2.

Electron emission of $5 \times 10^{-5} \text{ mol l}^{-1}$ estrone in an air-free mixture of 40% water and 60% ethanol as a function of the absorbed UV-quanta at 37 and 45°C. Inset I: $Q(e^-_{aq})$ values at the corresponding maxima. Inset II: pH-change of the media in dependence of the absorbed quanta.

Figure 3.

HPLC-chromatograms of $7.5 \times 10^{-5} \text{ mol l}^{-1}$ estrone in 40% water and 60% ethanol. (A) Dissolved in aerated media, unirradiated: (1) estrone, (2) products. (B) UV-irradiated after removing oxygen (UV-dose: $4 \times 10^{21} \text{ hv l}^{-1}$). (1) estrone remainder, (2) dimer, (3) unknown products. (C) Dissolved in air-free media, unirradiated (1) estrone. (D) Dissolved in air-free media and immediately irradiated (UV-dose $1.35 \times 10^{21} \text{ hv l}^{-1}$); (1) estrone remainder (17%), (2) metabolite, (3) unknown products (83% estrone conversion).

Figure 4. Survival curves (N/N_0 -ratio) of MCF-7 cells in aerated media containing $4 \times 10^{-2} \text{ mol l}^{-1}$ ethanol (pH ~ 7.4) as a function of the absorbed dose (Gy): (A) buffer, (B) $2.5 \times 10^{-5} \text{ mol l}^{-1}$ estrone, (C) $2.5 \times 10^{-5} \text{ mol l}^{-1}$ PRG and (D) a mixture of estrone and PRG, $2.5 \times 10^{-5} \text{ mol l}^{-1}$ each, saturated with air before irradiation.

Figure 5. Survival curves (N/N_0 -ratio) of MCF-7 cells in a media containing $4 \times 10^{-2} \text{ mol l}^{-1}$ ethanol (pH ~ 7.4) as a function of the absorbed dose (Gy): (A) buffer, (B) $2.5 \times 10^{-5} \text{ mol l}^{-1}$ estrone, (C) $2.5 \times 10^{-5} \text{ mol l}^{-1}$ PRG and (D) mixture of estrone and PRG, $2.5 \times 10^{-5} \text{ mol l}^{-1}$ each; saturated with N_2O before irradiation.

Figure 6. Survival curves (N/N_0 -ratio) of MCF-7 cells in a media containing $4 \times 10^{-2} \text{ mol l}^{-1}$ ethanol (pH ~ 7.4) as a function of the absorbed dose (Gy): (A) buffer, (B) $2.5 \times 10^{-5} \text{ mol l}^{-1}$ estrone, (C) $2.5 \times 10^{-5} \text{ mol l}^{-1}$ PRG and (D) mixture of estrone and PRG, $2.5 \times 10^{-5} \text{ mol l}^{-1}$ each; saturated with argon before irradiation.

Figure 7.

Microscopic pictures of MCF-7 cells in aerated buffer containing $4 \times 10^{-2} \text{ mol l}^{-1}$ ethanol. (A) unirradiated, (B) in the presence of $2.5 \times 10^{-5} \text{ mol l}^{-1}$ estrone, absorbed dose: 16.5 kGy, (C) in the presence of $2.5 \times 10^{-5} \text{ mol l}^{-1}$ PRG, absorbed dose: 16.5 kGy, (D) in the presence of a mixture of $1.25 \times 10^{-5} \text{ mol l}^{-1}$ estrone and PRG, respectively; absorbed dose: 16.5 kGy. Remark: The successive action on the cells under irradiation by estrone (B) and PRG (C) is best expressed by image (D), where the effect of both hormones is combined. These observations are in unison with the data shown in Figure 4.

Table I

$Q(e_{aq}^-)$ -values of various estrone concentrations at 37 and 45°C resulting of UV-irradiation ($\lambda = 254$ nm).

Series	Estrone (mol l ⁻¹)	$Q(e_{aq}^-)/37^\circ\text{C}$ at the peaks	$Q(e_{aq}^-)/45^\circ\text{C}$ at the peaks
I.	1×10^{-5}	(A) 2.17×10^{-2}	(A) 3.34×10^{-2}
		(B) 0.28×10^{-2}	(B) 0.36×10^{-2}
	2.5×10^{-5}	(A) 1.89×10^{-2}	
		(B) 0.20×10^{-2}	
		(C) 0.11×10^{-2}	
	5×10^{-5}	(A) 1.10×10^{-2}	(A) 1.90×10^{-2}
		(B) 0.13×10^{-2}	(B) 0.41×10^{-2}
		(C) 0.05×10^{-2}	(C) 0.27×10^{-2}
	7.5×10^{-5}	(A) 1.00×10^{-2}	
		(B) 0.11×10^{-2}	
		(C) 0.03×10^{-2}	
II.	7.5×10^{-5}	(A) 0.80×10^{-2}	
		(B) 0.06×10^{-2}	
		(C) 0.01×10^{-2}	

Solvent: 40% water and 60% ethanol. Series I: Substrate dissolved and UV-irradiated in air-free media. Series II: Substrate dissolved in aerated media, but UV-irradiated in air-free media.