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RAPID COMMUNICATION

Effects of estradiol and progesterone on the proinflammatory cytokine production by mononuclear cells from patients with chronic hepatitis C

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Abstract

AIM: To investigate the effects of estradiol (E2) and progesterone on the unstimulated and oxidative stress-stimulated production of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-8, and macrophage chemotactic protein (MCP)-1 by peripheral blood mononuclear cells (PBMCs) from patients with chronic hepatitis C and healthy controls.

METHODS: The PBMCs were separated from age-matched 72 males and 71 females with and without chronic hepatitis C, who were divided into two groups based on a mean menopausal age of 50 years. Oxidative stress was induced by hydrogen peroxide in the cells incubated in serum-free media. Cytokines in the culture supernatant were measured by an enzyme-linked immunosorbent assay.

RESULTS: The highest levels of the spontaneous production of TNF-α, IL-1β, IL-8, and MCP-1 by the unstimulated PBMCs were in the older male patients with chronic hepatitis C and the lowest levels were in the pre-menopausal female healthy controls. E2 inhibited the cytokine production by the unstimulated PBMCs from the older male and post-menopausal female patients, which was further stimulated by progesterone. The exposure to hydrogen peroxide in the PBMCs from the younger male and pre-menopausal female healthy subjects induced the production of cytokines. The change rates of the hydrogen peroxide-stimulated cytokine production were suppressed by E2 and enhanced by progesterone.

CONCLUSION: These findings suggest that E2 may play a favorable role in the course of persistent liver injury by preventing the accumulation of monocytes-macrophages and by inhibiting proinflammatory cytokine production, whereas progesterone may counteract the favorable E2 effects.

Key words: Estradiol; Progesterone; Mononuclear cell; Proinflammatory cytokine; Chemokine

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INTRODUCTION

In inflammatory and oxidative liver injury, the accumulation of leukocytes and macrophages including Kupffer cells on the sites of injury and inflammation in the liver is thought to be mediated by such cytokines as chemokines, including interleukin (IL)-8 and macrophage chemotactic protein (MCP)-1. These monocytes and macrophages are in turn

able to release proinflammatory cytokines such as tumor necrosis factor (TNF)-α and IL-1β, thus leading to the occurrence of persistent liver injury. It has been reported that chronic hepatitis C virus (HCV) infection tends to progress more rapidly in men than in women[1,2], and that the decline in estradiol (E2) production with menopause is associated with a spontaneous increase in TNF-α and IL-1β[3]. It should be noted that large increases in the amount of reactive oxygen species (ROS) lead to disturbance of prooxidant-antioxidant balance, or oxidative stress. Our previous studies have shown that hepatocytes possessed estrogen receptor (ER), and that in cultured hepatocytes in a state of oxidative stress, E2 inhibited the activation of the nuclear factor κB (NF-κB), a key transcription factor that induces multiple genes in response to inflammation and oxidative stress[4], through ER[5,6]. These findings suggest that E2 could enhance the anti-inflammatory activity in the injured liver by decreasing the proinflammatory cytokine production, and it might, therefore, play a cytoprotective role through ER in the liver[7,8]. However, regarding E2 and another female sex steroid, progesterone, there is little information about the direct effects of these sex hormones on the production of chemokines and proinflammatory cytokines by monocytes and macrophages in chronic HCV infection, although *in vitro* experiments in which monocytes were incubated with sex hormones have revealed conflicting results regarding monocyte cytokine production[9]. Therefore, this study investigated the effects of E2 and progesterone on the production of TNF-α, IL-1β, IL-8, and MCP-1 by the heparinized peripheral blood mononuclear cells (PBMCs), after stimulation with the ROS, hydrogen peroxide[10]. These effects were then compared between the blood samples from the age-matched male and female patients with chronic hepatitis C and the healthy controls, which were divided into two groups based on a mean menopausal age of 50 years.

MATERIALS AND METHODS

Patients

This study was conducted at Tokushima University Hospital and the subjects consisted of age-matched males (n = 72) and females (n = 71) including 71 patients with chronic hepatitis C and 72 healthy controls who were divided into younger, or pre-menopausal (< 50 years of age), and older, or post-menopausal (≥ 50 years of age) groups. The chronic hepatitis patients met the following criteria: a persistently elevated serum alanine aminotransferase (ALT, normal serum levels of ALT range between 5 and 40 U/L) level for at least six months; HCV-RNA seropositivity; seronegativity for Hepatitis B virus (HBV) surface antigen; exclusion of all other potential cause of chronic liver disease such as autoimmune hepatitis, primary biliary cirrhosis, drug-induced hepatitis, or metabolic liver disease; and no history of alcohol abuse, defined as an alcohol intake of more than 20 g per day over the previous year. In addition, any individuals taking oral contraceptives or corticosteroids, who had previously undergone an operation on the ovaries were also excluded from the study.

All females in the younger groups with and without chronic HCV infection had a normal ovarian cycle, and the mean ovarian cycle length was 28 d with a range of 26 to 32 d. Ethical approval was obtained from the Tokushima University Hospital ethics committee, and informed consent was obtained from all patients taking part in the study.

The heparinized peripheral blood samples were obtained in the morning after an overnight fast. In the pre-menopausal females, the blood samples were taken during the luteal phase 5 to 8 d before the onset of menses. The human PBMC fractions were separated using density-gradient centrifugation with a lymphocyte separation medium (Organon Teknika, Durham, NC, USA). After three washes with phosphate-buffered saline and buffered RPMI 1640 supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 1% L-glutamine (RPMI culture medium), the mononuclear cells were suspended at a concentration of 1×10^6/mL in RPMI culture medium with 10% heat-inactivated fetal bovine serum. The cells were cultured at 37℃ in a 5% CO_2 atmosphere and 100% humidity for 24 h and then treated with and without 17β-E2 (Sigma, St Louis, MO, USA) or progesterone (Wako, Osaka, Japan) for another 6 h in the presence and absence of the estrogen receptor antagonist, ICI-182, 780 (ICI: Tocris Cookson, Ballwin, MO, USA), or the PR antagonist RU486 (RU: Wako). In another experiment, the culture medium was changed with serum-free RPMI, and oxidative stress was induced by hydrogen peroxide (10^{-7}-10^{-5} mol/L). The cells were then incubated with either E2 or progesterone for up to 6 h in the presence and absence of ICI or RU. The steroid sex hormones and receptor antagonists were initially prepared as an ethanol stock solution (10^{-2} mol/L) and then were diluted with the culture medium in order to obtain an appropriate working solution concentration.

Cytokine and female sex hormone assays

The cytokines of TNF-α, IL-1β, IL-8 and MCP-1 and the female sex hormones of E2 and progesterone secreted into the culture supernatant were measured by an enzyme-linked immunosorbent assay (ELISA) using commercial kits (R&D Systems, Minneapolis, MN, USA) and by a radioimmunoassay using commercial kits (CIS Diagnostic, Tokyo, Japan), respectively, according to the manufacturer's instructions.

Statistical analysis

The data were presented as the mean ± SD, unless otherwise indicated. The means were compared between the two groups using the Wilcoxon's signed-rank test and the Mann-Whitney U test with Bonferroni correction. A P value of less than 0.05 was considered to be statistically significant.

RESULTS

Characteristics at the time of isolation of PBMCs from age-matched younger and older males and pre- and post-menopausal females of patients with chronic hepatitis C and healthy controls

As shown in Table 1, the background factors of 4 patient groups with chronic hepatitis C, such as the serum levels of

Table 1 Characteristics at the time of isolation of PBMCs from age-matched younger and older males and pre- and post-menopausal females of patients with chronic hepatitis C and healthy controls

	Patients with chronic hepatitis C				Healthy controls			
	Males (yr)		Females (yr)		Males (yr)		Females (yr)	
	< 50	⩾ 50	< 50	⩾ 50	< 50	⩾ 50	< 50	⩾ 50
n	18	18	17	18	18	18	18	18
Age (yr)	41 ± 7	69 ± 6	41 ± 7	68 ± 6	40 ± 6	68 ± 6	40 ± 6	69 ± 6
HCV-RNA (log copies/mL)	6.1 ± 0.8	6.5 ± 1.4	5.9 ± 0.8	6.4 ± 1.2	ND	ND	ND	ND
ALT (U/L)	118 ± 68	112 ± 52	105 ± 49	101 ± 55	27 ± 8	26 ± 8	24 ± 7	24 ± 8
Platelet count (10^3/mm^3)	208 ± 58	189 ± 52	206 ± 54	185 ± 55	230 ± 60	219 ± 53	225 ± 57	214 ± 58

The PBMCs were isolated from age-matched males (*n* = 72) and females (*n* = 71) of 71 patients with chronic hepatitis C and 72 healthy controls, who were divided into younger or pre-menopausal (< 50 years of age) and older or post-menopausal (⩾ 50 years of age) groups, based on a mean menopausal age of 50 years. The values are the mean ± SD (*n* = 17 or 18). ND: Not detected.

Table 2 Spontaneous production of TNF-α, IL-1β, IL-8, and MCP-1 by PBMCs from age-matched younger and older males and pre- and post-menopausal females of patients with chronic hepatitis C and healthy controls

Subjects with and without chronic HCV infection			TNF-α	IL-1β	IL-8	MCP-1
			(pg/mL supernatant)			
Males	Younger	Patients	354 ± 201[a]	253 ± 142	918 ± 499	403 ± 216
	(< 50 yr)	Controls	265 ± 161	180 ± 105	780 ± 420	308 ± 161
	Older	Patients	385 ± 234[a]	284 ± 160[a]	1087 ± 576[a]	461 ± 242[a]
	(⩾ 50 yr)	Controls	322 ± 200	217 ± 125	899 ± 480	364 ± 187
Females	Pre-menopausal	Patients	288 ± 177	198 ± 111	780 ± 410	334 ± 174
	(< 50 yr)	Controls	210 ± 126	167 ± 94	624 ± 335	275 ± 140
	Post-menopausal	Patients	314 ± 189	264 ± 149[a]	887 ± 473	387 ± 198
	(⩾ 50 yr)	Controls	241 ± 148	182 ± 100	699 ± 372	362 ± 201

The PBMCs were isolated from age-matched males (*n* = 72) and pre- and post-menopausal females (*n* = 71) of 71 patients with chronic hepatitis C and 72 healthy controls. The levels of TNF-α, IL-1β, IL-8, and MCP-1 in the culture supernatant were detected by means of an ELISA. The values are the mean ± SD (*n* = 17 or 18). [a]$P < 0.05$ *vs* the pre-menopausal healthy controls.

ALT and HCV-RNA, and platelet counts (normal ranges between 150 and 350 × 10^3/mm^3), were not significantly different among the younger (< 50 years of age), or pre-menopausal, and the older (⩾ 50 years of age), or post-menopausal groups. There was no significant difference of platelet counts between the patient groups and healthy control groups. Because platelet counts in chronic hepatitis C patients have been reported to be an indicator of the degree of hepatic fibrosis[11], most of the patients in this study seemed to show mild hepatic fibrosis.

Comparison of the spontaneous production of TNF-α, IL-1β, IL-8, and MCP-1 by PBMCs from age-matched younger and older males and pre- and post-menopausal females of patients with chronic hepatitis C and healthy controls

PBMCs were isolated from age-matched younger and older males and pre- and post-menopausal females of the patients with chronic hepatitis C and the healthy controls. In the premenopausal females, the blood samples were taken during the luteal phase of the ovarian cycle. The 24-h cultured PBMCs released TNF-α, IL-1β, IL-8, and MCP-1 into the culture medium (Table 2). The levels of E2 and progesterone in the culture supernatant were found to be under 10^{-12} mol/L. Although the net cytokine levels were considerably different among the individuals, the spontaneous production levels of cytokines in the unstimulated PBMCs appeared to show different tendencies between the 8 subgroups, the highest levels were present in the older male patients and the lowest levels were found in the pre-menopausal female controls ($P = 0.039$ for TNF-α, $P = 0.018$ for IL-1β, $P = 0.013$ for IL-8, and $P = 0.011$ for MCP-1). The chronic hepatitis C patients showed higher production levels of TNF-α, IL-1β, IL-8, and MCP-1 as compared with the age-matched healthy controls, and the mean production levels of cytokines in the older groups were higher than those in the younger groups, while the female subjects tended to produce much less cytokines from the unstimulated PBMCs than did the age-matched male subjects (Table 2).

Effects of E2 and progesterone on spontaneous production of TNF-α, IL-1β, IL-8, and MCP-1 by unstimulated PBMCs from age-matched older male and post-menopausal female patients with chronic hepatitis C

We next investigated the effects of E2 and progesterone on the augmented production of TNF-α, IL-1β, IL-8, and MCP-1 by the unstimulated PBMCs from the age-matched older male and post-menopausal female patients with chronic hepatitis C. When the unstimulated PBMCs were cultured for another 6 h without female sex hormones or receptor antagonists, the mean percentages of each initial value for the cytokine production reached up to 110%-123% (Table 3). Whereas the treatment with E2 and progesterone in the unstimulated PBMCs for 6 h significantly affected the change rates of the cytokine production in a dose dependent manner, the percentages

Table 3 Effects of E2 and progesterone on spontaneous production of TNF-α, IL-1β, IL-8, and MCP-1 by unstimulated PBMCs from age-matched older male and post-menopausal female patients with chronic hepatitis C

Chronic hepatitis C subjects	TNF-α	IL-1β	IL-8	MCP-1
			(% of initial value)	
Older male patients				
None	123 ± 14	120 ± 16	119 ± 12	114 ± 15
+ E2	82 ± 10[a]	84 ± 10[a]	80 ± 11[a]	78 ± 11a
+ E2 + ICI	118 ± 16	114 ± 18	108 ± 19	99 ± 19
+ Progesterone	167 ± 19[a]	169 ± 20[a]	159 ± 18[a]	169 ± 28[a]
+ Progesterone + RU	129 ± 21	124 ± 21	132 ± 19	122 ± 24
Post-menopausal female patients				
None	118 ± 15	119 ± 14	122 ± 16	110 ± 13
+ E2	83 ± 11[a]	79 ± 9[a]	81 ± 12[a]	74 ± 11[a]
+ E2 + ICI	108 ± 14	122 ± 16	117 ± 19	104 ± 19
+ Progesterone	167 ± 13[a]	170 ± 24[a]	159 ± 20[a]	175 ± 30[a]
+ Progesterone + RU	130 ± 27	125 ± 28	132 ± 30	123 ± 25

The unstimulated PBMCs from the age-matched older male and post-menopausal female patients with chronic hepatitis C were cultured for another 6 h with and without 10^{-8} mol/L E2 (E2) or 10^{-7} mol/L progesterone (progesterone) in the presence and absence of 10^{-6} mol/L ICI (ICI) or 10^{-6} mol/L RU (RU). The levels of TNF-α, IL-1β, IL-8, and MCP-1 in the culture supernatant were detected by means of an ELISA. The results were expressed as the percentages of each initial value for the cytokine production in the absence of the female sex hormones and receptor antagonists. The values are the mean ± SD (n = 18). [a]$P < 0.05$ *vs* 6-h-cultures in the absence of the female sex hormones and receptor antagonists.

of each initial value decreased significantly in the PBMCs treated with E2 at 10^{-8} and 10^{-7} mol/L, and they increased significantly in the PBMCs treated with 10^{-7} mol/L progesterone (Figure 1). There was no significant difference between the change rates of the cytokine production in the male and female patients with chronic hepatitis C.

The inhibitory effects of 10^{-8} mol/L E2 on the unstimulated cytokine production in the male and female patients were blocked by the specific ER antagonist ICI at a dose of 10^{-6} mol/L, while the further enhancement effects of 10^{-7} mol/L progesterone on the unstimulated cytokine production in both genders were blocked by the PR antagonist RU (Table 3). The treatment with ICI or RU alone had no effect on any of the parameters examined herein (data not shown).

Effects of E2 and progesterone on hydrogen peroxide-stimulated production of TNF-α, IL-1β, IL-8, and MCP-1 by PBMCs from age-matched younger male and pre-m enopausal female healthy controls

The exposure to low doses of hydrogen peroxide (10^{-7}-10^{-5} mol/L) in the PBMCs from the age-matched younger male and pre-menopausal female healthy controls, incubated in serum-free RPMI for 6 h, was observed to stimulate the production of TNF-α, IL-1β, IL-8, and MCP-1 in a dose-dependent manner (data not shown). The subsequent studies used a dose of 10^{-5} mol/L of hydrogen peroxide for further stimulation of the incubated PBMCs. The exposure to hydrogen peroxide induced a time-dependent and transient cytokine production, peaking at 1-6 h, over a 6 h period (Figure 2). There was no significant difference between the cytokine levels in the male and female healthy controls. The cytokine levels in the culture supernatant (Figure 2) peaked after 6 h. Subsequent studies used an incubation time of 6 h to measure the levels of TNF-α, IL-1β, IL-8, and MCP-1 after the hydrogen peroxide exposure.

The hydrogen peroxide-stimulated cytokine production was inhibited by 10^{-8} mol/L E2 (Table 4). The inhibitory effect of E2 was blocked by 10^{-6} mol/L ICI (Table 4). In contrast to E2, progesterone treatment for 6 h resulted in the further cytokine production in the oxidative stress-stimulated PBMCs. The stimulatory effect of progesterone (10^{-7} mol/L) was blocked by 10^{-6} mol/L RU (Table 4). No parameters examined in the PBMCs were found to be significantly different between the male and female subjects.

DISCUSSION

In the present study, the highest levels of the spontaneous production of TNF-α, IL-1β, IL-8, and MCP-1 by the unstimulated PBMCs were found to be in the older male patients with chronic hepatitis C, and the lowest levels were in the pre-menopausal female healthy subjects, although the cytokine levels were considerably different among the individuals. The male subjects tended to produce cytokines from the unstimulated PBMCs to a much greater degree than did the age-matched female subjects. The augmented cytokine production by the PBMCs from the older male and post-menopausal female patients with chronic hepatitis C was inhibited by supplementation with E2, and was further stimulated by supplementation with progesterone through their receptors, when the unstimulated cells were cultured for an additional 6 h. The exposure to low doses of hydrogen peroxide in the PBMCs from younger male and pre-menopausal female healthy subjects incubated in the serum-free media for 6 h was observed to induce cytokine production. The change rates of the hydrogen peroxide-stimulated production of TNF-α, IL-1β, IL-8, and MCP-1 in the PBMCs were suppressed by E2, and were enhanced by progesterone through their receptors. The specificity of the E2-mediated anti-inflammatory induction through the ER and the progesterone-mediated proinflammatory induction through the PR was shown

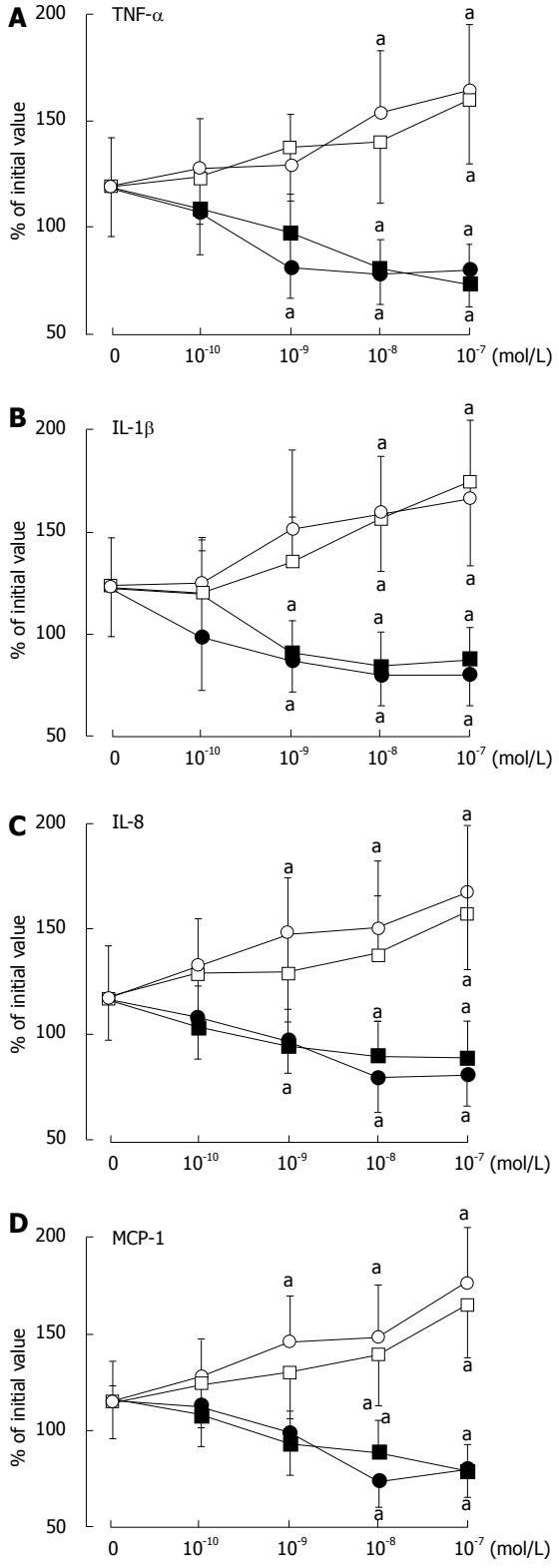

Figure 1 Effects of E2 and progesterone on spontaneous production of TNF-α (**A**), IL-1β (**B**), IL-8 (**C**), and MCP-1 (**D**) by unstimulated PBMCs from age-matched older male and post-menopausal female patients with chronic hepatitis C. The unstimulated PBMCs from the age-matched older male (black square) and post-menopausal female (black circle) patients with chronic hepatitis C were cultured for another 6 h with and without E2 (10^{-10}-10^{-7} mol/L) (solid) or progesterone (10^{-10}-10^{-7} mol/L) (open). The spontaneous production levels of TNF-α (**A**), IL-1β (**B**), IL-8 (**C**), and MCP-1 (**D**) in the culture supernatant were detected by means of an ELISA. The results were expressed as the percentages of each initial value for the cytokine production in the absence of the female sex hormones. The values are the mean ± SD (n = 18). $^{a}P < 0.05$ in comparison to the 6-h-cultures in the absence of the female sex hormones.

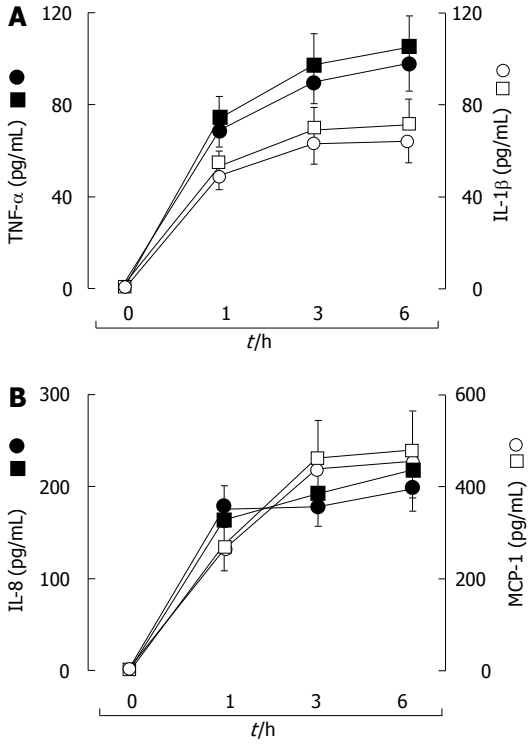

Figure 2 Stimulation of TNF-α, IL-1β (**A**), IL-8, and MCP-1 (**B**) production after exposure to hydrogen peroxide by unstimulated PBMCs from age-matched younger male and pre-menopausal female healthy controls. The unstimulated PBMCs from the age-matched younger male (black square) and pre-menopausal female (black circle) healthy controls were incubated for up to 6 h in serum-free RPMI in the presence of 10^{-5} mol/L hydrogen peroxide. The levels of TNF-α (**A** solid), IL-1β (**A** open), IL-8 (**B** solid), and MCP-1 (**B** open) in the culture supernatant were detected by means of an ELISA. The values are the mean ± SD (n = 10).

by ICI and RU, respectively, in both the unstimulated and oxidative stress-stimulated PBMCs. The inhibitory effect of E2 at a dose of 10^{-8} mol/L on the unstimulated and stimulated cytokine production was blocked by ICI in both gender subjects. Treatment with the progesterone receptor antagonist RU led to a blockage of further cytokine production induced with 10^{-7} mol/L progesterone by the unstimulated and stimulated PBMCs from both genders. No parameters examined in the PBMCs were found to be significantly different between the male and female subjects.

There is a large body of evidence indicating that the decline in the ovarian function with menopause is associated with spontaneous increases in TNF-α, IL-1β, and IL-6[3]. E2, at physiological concentrations (10^{-11}-10^{-8} mol/L), has been reported to inhibit the spontaneous secretion of these proinflammatory cytokines in whole blood cultures[12] or PBMCs[13]. The unstimulated production of TNF-α and IL-1β in PBMCs has been reported to be higher in patients with chronic hepatitis C than in healthy subjects[14]. These findings were consistent with the present data. The *in vivo* treatment with E2 transdermally in postmenopausal women has been reported to decrease the spontaneous IL-6 production by PBMCs after 12 mo of the therapy[13]. One preliminary study also showed the hydrogen peroxide-induced TNF-α

Table 4 Effects of E2 and progesterone on hydrogen peroxide-stimulated production of TNF-α, IL-1β, IL-8, and MCP-1 by PBMCs from age-matched younger male and pre-menopausal female healthy controls

Healthy subjects	TNF-α	IL-1β	IL-8	MCP-1
		(pg/mL supernatant)		
Younger male controls				
Oxidative stress	102 ± 17	66 ± 11	218 ± 41	474 ± 81
Oxidative stress + E2	78 ± 11[a]	49 ± 6[a]	174 ± 27[a]	332 ± 49[a]
Oxidative stress + E2 + ICI	107 ± 10	62 ± 11	223 ± 46	457 ± 87
Oxidative stress + Progesterone	140 ± 14[a]	86 ± 9[a]	289 ± 40[a]	658 ± 98[a]
Oxidative stress + Progesterone + RU	110 ± 23	75 ± 14	223 ± 32	516 ± 73
Pre-menopausal female controls				
Oxidative stress	91 ± 15	57 ± 9	195 ± 37	435 ± 85
Oxidative stress + E2	64 ± 11[a]	42 ± 6[a]	155 ± 29[a]	314 ± 68[a]
Oxidative stress + E2 + ICI	95 ± 13	54 ± 9	187 ± 37	419 ± 92
Oxidative stress + Progesterone	129 ± 12[a]	75 ± 12[a]	255 ± 40[a]	586 ± 70[a]
Oxidative stress + Progesterone + RU	101 ± 19	63 ± 11	208 ± 40	462 ± 73

The unstimulated PBMCs from the age-matched younger male (n = 18) and pre-menopausal female (n = 18) healthy controls were incubated for up to 6 h in serum-free RPMI after exposure to 10^{-5} mol/L hydrogen peroxide (oxidative stress) with and without 10^{-8} mol/L E2 (E2) or 10^{-7} mol/L progesterone (Progesterone) in the presence and absence of 10^{-6} mol/L ICI (ICI) or 10^{-6} mol/L RU (RU). The levels of TNF-α, IL-1β, IL-8, and MCP-1 in the culture supernatant incubated for 6 h were detected by means of an ELISA. The values are the mean ± SD (n = 18). [a]$P < 0.05$ *vs* 1 h cultures for MCP-1 or 6 h cultures for TNF-α, IL-1β, and IL-8 after hydrogen peroxide exposure in the absence of the female sex hormones and receptor antagonists.

and MCP-1 expressions to be attenuated by E2 in the peritoneal macrophages of female mice[15]. Furthermore, E2 is able to attenuate IL-1β in ER expressing HepG2 cells[16], and to ameliorate the burn-induced increase in the serum TNF-α levels in rats[17]. These findings suggest that E2 may exert a hepatoprotective action against inflammation and oxidative stress, at least in part, by preventing accumulation of monocytes and macrophages and by inhibiting the production of proinflammatory cytokines.

As far as the *in vitro* studies with female sex hormones on the production of TNF-α and IL-1β by monocytes are concerned, however, conflicting data have been published[9], varying from some[18-20] to no[12,21] effect of E2 or progesterone on cytokine production. E2 has been reported to suppress the TNF-α production in unstimulated PBMCs, but not in endotoxin-stimulated PBMCs, from postmenopausal females with osteoporosis[22]. An inhibition of IL-1β production in endotoxin-stimulated monocytes by E2 or progesterone at physiological concentrations has also been reported[23]. The results of the reported studies did not correlate with the present data. These conflicting results may possibly be due to the handling of the cells during the *in vitro* research, different experimental methods used, and/or differences in the subjects employed in the studies.

In the premenopausal female subjects with and without chronic hepatitis C enrolled herein, the blood samples were taken during the luteal phase of the menstrual cycle. During the luteal phase, the serum concentration of endogenous progesterone rises up to a maximum of about 10^{-7} mol/L, which can be ten to a hundred times higher than E2. Higher blood levels of TNF-α have been observed during the luteal phase in comparison to the follicular phase[24]. In males, a higher percentage of IL-1β producing stimulated monocytes has been demonstrated in comparison to females in the follicular phase[19]. The male sex hormone testosterone has some structural and functional similarities to progesterone[25]. Judging from these findings and the present data showing that treatment with E2 (10^{-8} and 10^{-7} mol/L) and progesterone (10^{-7} mol/L) significantly affected the change rate of the cytokine production in hydrogen peroxide-stimulated PBMCs, E2 may, therefore, exert an anti-inflammatory action against both inflammation and oxidative stress in the mononuclear cells from the chronic hepatitis C patients, whereas progesterone may counteract the favorable effects of E2.

HCV infections are recognized to be a major causative factor in the development of liver injury leading to cirrhosis[26,27] The HCV core protein has been reported to enhance the signaling pathway of NF-κB activation in human hepatoma HuH-7 and cervical cancer HeLa cells, and the HCV core protein is triggered by TNF-α-related cytokines[28]. Damage to the parenchymal cell membranes and liver mitochondria could produce ROS derived from lipid peroxidative processes, which constitute a general feature of a sustained inflammatory response and liver injury[29]. In comparison to other types of ROS, hydrogen peroxide is more stable and membrane permeable leading to the hypothesis that it acts as a second messenger in regulating the signaling events, including the mitogen-activated protein kinase (MAPK) activation. We have already reported that E2 inhibited the prooxidant-induced lipid peroxidation in rat liver mitochondria[5], attenuated ROS generation and NF-κB activation in cultured rat hepatocytes in a state of prooxidant-induced oxidative stress[6], while also suppressing the hydrogen peroxide-induced activation of MAPKs and transcription factors including NF-κB in cultured rat hepatic stellate cells[30]. In the present study, hydrogen peroxide exposure resulted in an increase in the TNF-α, IL-1β, IL-8, and MCP-1 levels in the cultured mononuclear cells from the male and female healthy subjects. The oxidative stress-stimulated cytokine expression was attenuated by E2 and augmented by progesterone in a dose-dependent manner without any significant difference between the males and females. These effects of E2 and progesterone were blocked by

their receptor antagonists ICI and RU, indicating that ER and PR could mediate female sex hormone action in the oxidative stress-stimulated monocytes and macrophages.

Finally, the current data suggest that E2 may play a favorable role in the course of persistent liver injury, at least in part, by preventing the accumulation of monocytes and macrophages and by also inhibiting the proinflammatory cytokine production through ER, whereas progesterone may counteract these positive E2 effects by enhancing the accumulation of inflammatory cells and their cytokine production through PR.

COMMENTS

Background

Parenchymal cell membrane damage could produce reactive oxygen species (ROS) derived from lipid peroxidative processes, which represent the general feature of sustained inflammatory response and liver injury. A chronic hepatitis C virus infection tends to progress more rapidly in men than women. There is little information about the effects of estradiol (E2) and progesterone on the proinflammatory cytokine production by prooxidant-stimulated monocytes in chronic hepatic C patients.

Research frontiers

We have recently hypothesized that E2 could play a cytoprotective role in the injured liver by inhibiting the ROS generation, antioxidant enzyme loss, and induction of redox sensitive transcription factors.

Innovations and breakthroughs

This study focused on comparison of the oxidative stress-stimulated proinflammatory cytokine production between younger and older patients, who were divided into two groups based on a mean menopausal age of 50 years, demonstrating that E2 might play a favorable role in the course of persistent liver injury, whereas progesterone might counteract the favorable E2 effects.

Applications

The favorable activity of E2 may be involved in the persistent liver injury of different liver diseases such as alcoholic and non- alcoholic liver diseases as well as chronic hepatitis C and B.

Peer review

The study is of particular interest because the role and importance of the sex hormones in the regulation of some cell functions during disturbance in the prooxidant-antioxidant balance is understood. It is very important that study links clinical and experimental part.

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