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SHORT COMMUNICATION

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The effects of UVB and arsenic and their interaction on β_2-adrenergic receptors in cultured keratinocytes

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Epidemiological studies have revealed that exposure to arsenic (As) induces skin cancer [1]. As-induced skin cancers are usually found on non-sun-exposed areas [2]. Ultraviolet B (UVB) irradiation has been used in the treatment of hyperproliferative dermatoses, such as psoriasis [3] and cutaneous T-cell lymphoma [4]. Furthermore, UVB irradiation exerts an inhibitory effect on proliferation of As-induced Bowen's disease [5]. Thus, UVB irradiation may play a modulatory role in As carcinogenesis.

Epidermal keratinocytes contain the β-adrenergic adenylate cyclase system, which upon activation causes an accumulation of intracellular cyclic adenosine monophosphates (cAMP) through stimulatory GTP-binding proteins (G-proteins) [6]. The β-adrenergic receptor (β-AR) is responsible for selective recognition and binding of catecholamines, affecting epidermal cell proliferation and differentiation [7]. The responsiveness of this receptor system has been found to be defective in skin diseases such as psoriasis [8]. Our previous findings have revealed that UVB irradiation or As exposure can suppress β_2-AR expression in cultured keratinocytes [9, 10]. In the present study we further examined the effects of the interaction of UVB and As on the expression of β_2-AR and its responsiveness by measuring the difference in cAMP contents after β_2 agonistic stimulation.

The keratinocytes were obtained from adult foreskins from routine circumcisions. They were incubated in serum-free medium according to a previously described method [11]. Keratinocytes at the third passage were treated with various regimens of sodium arsenite (1 μ*M*) and/or UVB (50 mJ/cm^2) as follows: (1) no treatment (control treatment); (2) incubated with As for 48 h (As treatment); (3) irradiated with UVB after a 48-h incubation (UVB treatment); (4) incubated with As for 24 h then irradiated with UVB, followed by a 24-h incubation in culture medium (As-UVB treatment); (5) irradiated with UVB then incubated for 24 h in culture medium, followed by a 24-h As exposure (UVB-As treatment); (6) irradiated with an initial 12.5 mJ/cm^2 UVB, then immediately exposed to As for 24 h, again irradiated with UVB followed by a 24-h incubation in culture medium (UVB-As-UVB treatment).

Cell extracts were prepared by ultrasonication followed by ultracentrifugation. A modification of the β-AR assay developed by Steinkraus et al. [12] was used to determine the binding of [^{125}I]CYP to keratinocyte membranes. Specific binding was calculated from the difference between total and nonspecific binding in the presence of 0.1 m*M* (±)-propanolol. The β-AR density (B_{max}) and dissociation constant (K_d) for [^{125}I]CYP binding were determined from the saturation curves of specific binding analyzed by the Scatchard method. The cAMP levels at baseline and after agonistic stimulation were determined using a commercial radioimmunoassay kit. Protein was measured according to the method of Lowry et al. using bovine serum albumin as a standard.

Keratinocytes treated with As and UVB at 1 μ*M* and 50 mJ/cm^2, respectively, exhibited no apparent cytotoxicity as revealed by the XTT cell viability assay [13] (data not shown). The β_2-AR density and cAMP levels of keratinocytes treated with As and UVB are shown in Table 1. The value of maximal binding capacity (B_{max}) reflects the average β_2-AR density on the cells. The B_{max} values resulting from the As (53.2 ± 8.3 fmol/mg protein), UVB

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Table 1 Effects of As and UVB on the expression and responsiveness of β_2-AR. Cultured human keratinocytes treated with As and UVB in different combinations were assessed in terms of β_2-AR expression and its responsiveness. The maximal binding capacity (B_{max}) and binding affinity (K_d, dissociation constant) were calculated by the Scatchard method. The responsiveness of this adenylate cyclase system was determined by the difference between basal cAMP concentrations and levels following stimulation with an agonistic agent (10 μ*M* isoproterenol). The differences between groups were subjected to one-way analysis of variance. Values are means ± SD (n = 3)

Treatment	β_2-AR B_{max} (fmol/mg protein)	K_d (p*M*)	c-AMP (pmol/mg protein)	
			Basal	Stimulated
Control	92.9 ± 10.3	55.2 ± 5.1	1.9 ± 0.3	46.5 ± 8.1
As	53.2 ± 8.3*	59.3 ± 6.1	1.8 ± 0.5	23.2 ± 5.6*
UVB	44.8 ± 7.2*	50.8 ± 5.2	1.8 ± 0.3	18.3 ± 7.5*
As-UVB	47.8 ± 7.2*	53.1 ± 7.9	1.7 ± 0.3	20.8 ± 4.1*
UVB-As	99.3 ± 11.5	54.3 ± 4.1	2.0 ± 0.4	51.9 ± 9.4
UVB-As-UVB	69.2 ± 6.4*	55.7 ± 8.4	1.9 ± 0.4	30.6 ± 6.3*

* $P < 0.001$ vs control

(44.8 ± 7.2 fmol/mg protein) and As-UVB (47.8 ± 7.2 fmol/mg protein) treatments were significantly lower than those resulting from the control treatment (92.9 ± 10.3 fmol/mg protein; $P < 0.001$). On the other hand, there was no significant difference between the B_{max} values resulting from the control and the UVB-As (99.3 ± 11.5 fmol/mg protein) treatments. To clarify the role of UVB in suppressing β_2-AR, we added an initially lower dose (12.5 mJ/cm^2) of UVB radiation to the As-UVB treatment for confirmation of its biological effect. The expression of β_2-AR in keratinocytes receiving the UVB-As-UVB treatment (69.2 ± 6.4 fmol/mg protein) ranged between the expression levels of those receiving the control and the As-UVB treatments (Table 1). These findings suggest that UVB pretreatment prevents the β_2-AR density decrease due to As exposure. The binding affinities of β_2-AR were determined by calculating the K_d constant following each treatment. No significant differences were noted in the K_d values among the various treatments, suggesting that these treatments do not influence the binding affinity of β_2-AR, i.e. the structure of the β_2-AR protein remains intact.

Both basal and stimulated levels of intracellular cAMP were investigated in terms of the responsiveness of the β_2-adrenergic adenylate cyclase system. The basal levels of intracellular cAMP (without agonistic stimulation) following all experimental and control treatments showed no significant differences. In contrast, stimulation with β_2-AR agonist (10 μ*M* isoproterenol) resulted in significantly reduced levels of intracellular cAMP following the As (23.2 ± 5.6 pmol/mg protein), UVB (18.3 ± 7.5 pmol/mg protein), As-UVB (20.8 ± 4.1 pmol/mg protein) and UVB-As-UVB (30.6 ± 6.3 pmol/mg protein) treatments as compared with that following the control treatment (46.5 ± 8.1 pmol/mg protein; $P < 0.001$). In parallel with the β_2-AR density results, there were no significant differences in stimulated levels of intracellular cAMP between the control and UVB-As (51.9 ± 9.4 pmol/mg protein) treatments (Table 1). The cAMP levels and receptor density in keratinocytes receiving the same treatment were correlated, indicating again that As and UVB treatments do not induce alterations in the structure of β_2-AR.

It has been suggested that the β-adrenergic adenylate cyclase-cAMP system in epidermal tissue might play a crucial role in proliferative and differentiative homeostasis. Iizuka et al. [14] have reported that decreased responsiveness of β-adrenergic adenylate cyclase in psoriatic epidermis is probably due to the defective structure of β-AR or its inability to couple with G-proteins. In addition, Schallreuter et al. [15] have found that the adenylate cyclase of human keratinocytes is sensitive to agents that stimulate the ARs, and this may be a factor in the regulation of epidermal growth. cAMP has long been regarded as a second messenger and a regulator of human keratinocyte proliferation. cAMP inhibits keratinocyte proliferation in vitro at high concentrations or that induced by treatment with forskolin or phosphodiesterase inhibitors, such as isobutylmethylxanthine [16]. In addition, isoproterenol, a β_2-AR agonist, selectively activates adenylate cyclase and increases the levels of the differentiation markers keratins K1 and K10, involucrin and transglutaminase [17]. The molecular mechanisms involved in the regulation of keratinocyte differentiation are not yet fully understood.

There are two factors which may contribute to the responsiveness of the β-adrenergic adenylate cyclase-cAMP system: first is the receptor density and its binding affinity at the membrane receptor level; second is the catalytic function of adenylate cyclase confined to the inner cytoplasmic membrane. In this study, we demonstrated that As and UVB treatments induced no alterations in either the structure or the binding affinity of β_2-AR, but only in the receptor density. Spiegel et al. have shown that As may irreversibly disturb the catalytic function of adenylate cyclase by binding to the vicinal sulfhydryl group at its active site [18]. Intracellular SH-containing antioxidant enzymes, such as glutathione peroxidase, glutathione reductase, superoxide dismutase, thioredoxin reductase, catalase and others, respond to UV radiation [19, 20] and protect against UV and As damage [21]. UVB pretreatment stimulates the intracellular redox system and retards the formation of intramolecular disulfide bonds induced by As. Therefore, the increase in redox elements resulting from UVB treatment may contribute to the prevention of irreversible damage by subsequent addition of As. This mechanism may partially explain why a stable cAMP level was found following the UVB-As treatment as compared to the other treatments.

In the present study, using β_2-AR expression and its responsiveness, i.e. β_2-AR density and cAMP as indicators, we investigated the effects of UVB and As and their interactions on keratinocyte proliferation and, possibly, differentiation. The β_2-AR densities and cAMP levels significantly decreased in response to four experimental treatments, i.e. As, UVB, As-UVB and UVB-As-UVB treatments, but not the UVB-As treatment. These results sug-

gest that UVB pretreatment prevents the inhibitory effects of As on the β-adrenergic adenylate cyclase system. The different responses to the As-UVB and UVB-As treatments indicates that there may be different mechanism(s) and interactions of UVB and As in keratinocytes. The controversy as to whether UVB could play a prohibitory role in the process of As-induced carcinogenesis has long been debated [2, 7, 22, 23]. Our results may provide a new direction for studying the interaction of UVB and As in the process of carcinogenesis.

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