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Brief Communication

Upregulation of Nitric Oxide Synthase Activity by All-*trans* Retinoic Acid and 13-*cis* Retinoic Acid in Human Malignant Keratinocytes

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Effect of retinoids, *i.e.*, all-*trans* retinoic acid and 13-*cis* retinoic acid, on the activity of nitric oxide synthase (NOS) was evaluated in human malignant keratinocytes to examine the possible correlation of retinoids with NOS activities. All-*trans* retinoic acid and 13-*cis* retinoic acid did not alter the nitric oxide (NO) production. However, in the presence of lipopolysaccharide (LPS, 1 μg/mL), they significantly increased NO release in a dose-dependent manner until 48 h at concentrations of 50~100 μM. The degree of upregulation of NO by all-*trans* retinoic acid and 13-*cis* retinoic acid increased up to 35% and 37%, respectively, compared to that by the control, which demonstrated the upregulation of LPS-inducible nitric oxide synthase (iNOS)-dependent generation of NO as well as showing a crucial link between retinoids-induced activity and NOS. Findings of this study now suggest that the upregulation of LPS-iNOS activity may be associated with modulation of retinoids-induced control of cellular developmental processes, which may produce new therapeutics of retinoids in the complexity of how NO affects human keratinocytes.

Key Words: All-*trans* retinoic acid, 13-*cis* retinoic acid, Inducible nitric oxide synthase, Nitric oxide, Human malignant keratinocytes

Monitoring changes of specific enzyme activity can provide a precise description of the progression of cellular proliferation, differentiation, and apoptosis that can lead to better understanding of potential mechanisms involved in the development as well as the treatment of various cancers.

Retinoids are natural and synthetic derivatives of vitamin A. They are potential regulators of cellular activities, including cell growth, differentiation and apoptosis (Altucci and Gronemeyer, 2001; Rudkin et al., 2002; Darwiche et al., 2005; Ertesvag et al., 2009). The clinical trials and results of retinoids in the treatment and prevention of skin cancer and their potential use as immunomodulators or tumor-suppressive agents have been described (Niles, 2002; Carratu et al., 2012).

Nitric oxide synthase (NOS) is a well-known enzyme marker associated with cell growth and development through production of nitric oxide (NO). NO or NOS levels have both positive and negative effects, because they have been implicated in both promoting and preventing cancer-related events such as angiogenesis, apoptosis, cell cycle, invasion, metastasis, and carcinogenesis (Lechner et al., 2005; Hickok and Thomas, 2010; Ridnour et al., 2008; Moon, 2011, 2018).

The regulation of NOS enzyme activity associated with retinoids-mediated apoptosis and/or cellular proliferation and differentiation for human keratinocyte growth or development has not been reported yet.

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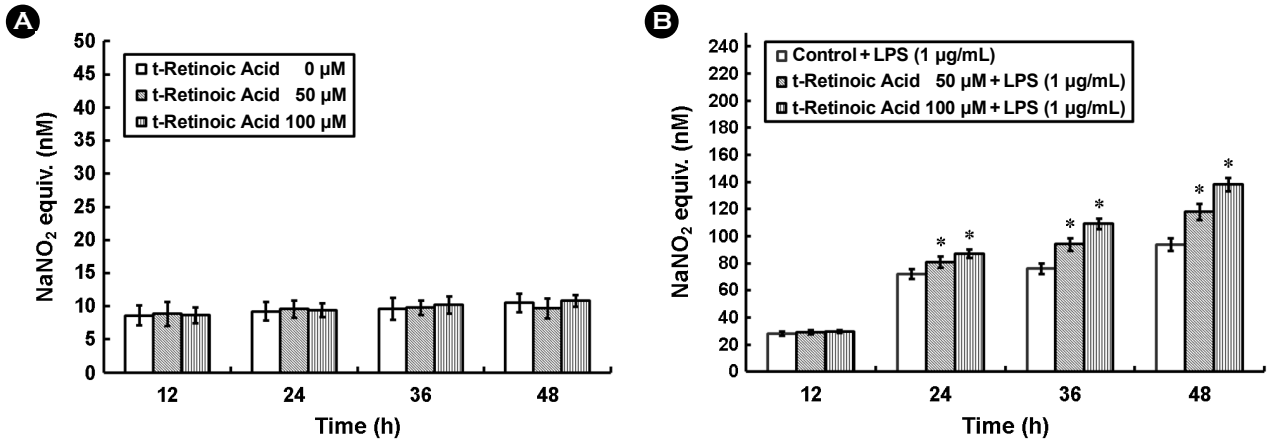

Fig. 1. Effect of all-*trans* retinoic acid on nitric oxide synthase activity in human malignant keratinocytes. (A) No regulatory effect of all-*trans* retinoic acid on nitric oxide synthase activity. (B) Dose-dependent upregulation of LPS-induced nitric oxide synthase activity by all-*trans* retinoic acid.

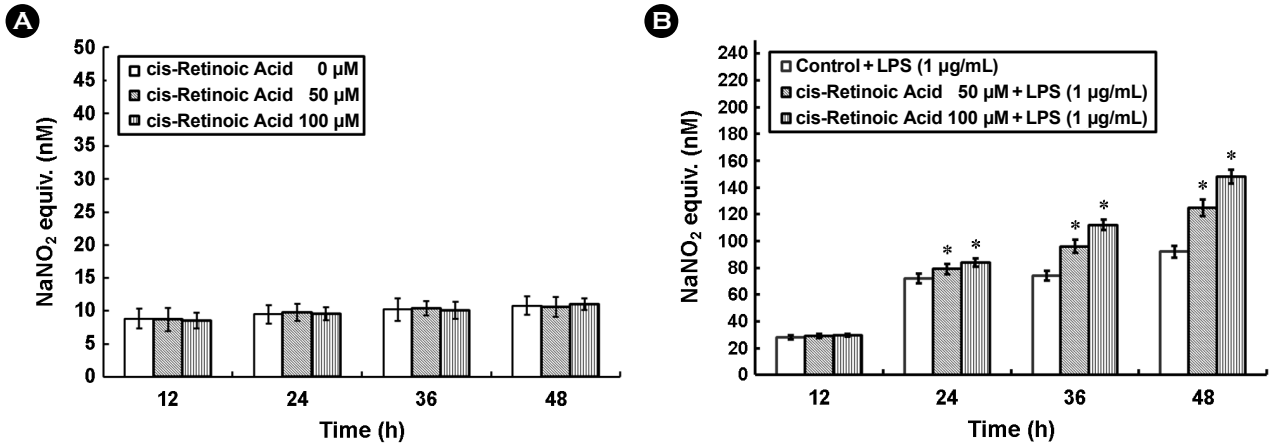

Fig. 2. Effect of 13-*cis* retinoic acid on nitric oxide synthase activity in human malignant keratinocytes. (A) No regulatory effect of 13-*cis* retinoic acid on nitric oxide synthase activity. (B) Dose-dependent upregulation of LPS-induced nitric oxide synthase activity by 13-*cis* retinoic acid. All-*trans* retinoic acid and 13-*cis* retinoic acid were added to the culture medium at 0 h of incubation and the NO values were measured at 12, 24, 36, and 48 h. Each value represents the mean ± S.E. of three independent determinations. No significant difference in the level of NO release was found between all-*trans* retinoic acid and 13-*cis* retinoic acid treated and control in the absence of LPS. A significant difference in LPS-induced NO productions between the control and all-*trans* retinoic acid and 13-*cis* retinoic acid treated samples was indicated by $*P<0.01$.

To investigate the possible correlation of cellular NOS activity with the generation of NO by retinoids, cultured malignant human epidermal keratinocytes were treated with retinoids, *i.e.*, all-*trans* retinoic acid and 13-*cis* retinoic acid, in the absence or presence of lipopolysaccharide (LPS). NO production was assessed by measuring nitrite in phenol-red and cell-free culture supernatant to represent the activity of NOS (Moon, 2011). There was no distinguishable difference in NO release between control and retinoids-treated cells in the absence of LPS (Fig. 1A and 2A). However, in the presence of LPS (1 μg/mL), treatment of human malignant keratinocytes with all-*trans* retinoic acid (100 μM) and 13-*cis* retinoic acid (100 μM) increased NO production up to 35% and 37% compared to the control, respectively (Fig. 1B and 2B). The degree of upregulation was increased in a dose-dependent manner until 48 h at concentrations of 50~100 μM (Fig. 1B and 2B), confirming that retinoids upregulated LPS-inducible nitric oxide synthase (iNOS)-dependent

generation of NO in human skin cells. This study demonstrates that human malignant keratinocytes (SCC-13) can secrete nitric oxide (NO) in response to LPS, consistent with previous results (Bécherel et al., 1995, 1997; Heck et al., 1992; Moon, 2011), and suggests that the changes of LPS-inducible keratinocytes NO synthase activity may be involved in the regulation of cell growth and development induced by retinoids.

The differences in the final biological outcomes of cellular NO effects has been described within a cell. Nitric oxide (NO) initiates apoptosis in various cells and is correlated with tumor suppression (Brüne et al., 1999; Jun et al., 1998; Messmer et al., 1995). Long-standing overproduction of NO can act as a proapoptotic modulator, and a high NO level has been proposed as being able to suppress metastasis (Aranda et al., 2012). In addition, upregulation of the iNOS gene can affect the suppression of tumorigenicity and metastasis of oral cancer cells (Harada et al., 2004). Cytokines (IL-1beta, IFN-gamma, and TNF-alpha)-inducible nitric oxide synthase (iNOS) inhibited proliferation of human MCF-7 breast cancer cells, showing increased NOS activity with a low proliferation rate (Reveneau et al., 1999). Recent study suggested that the downregulation of inducible NO generation was responsible for the *N*-nitroso-*N*-methylurea and *N*-nitroso-*N*-ethylurea-induced carcinogenicity in human epidermal keratinocytes (Moon, 2018).

In contrast, iNOS expression is correlated with cervical lymph node metastasis in oral squamous cell carcinoma (Chen et al., 2002). It was also reported that increased NO levels were manifested in the progression of endometrial angiogenesis and carcinogenesis (Cinel et al., 2002; Ozel et al., 2006).

Programmed cell death or apoptosis in murine macrophages by NO has been associated with differentiation of neuronal cells induced by all-*trans* retinoic acid (Messmer et al., 1995; Uruno et al., 2005). It has been reported that all-*trans* retinoic acid is a potent inhibitor of leukemia cell proliferation, and can induce differentiation of acute promyelocytic leukemia cells both *in vitro* and *in vivo* (Kambhampati et al., 2003). Especially, retinoids have influenced keratinocyte proliferation, differentiation, and keratinization (Beckenbach et al., 2015).

Findings of this study provide a crucial link between retinoids-induced activity and NOS activity, suggesting that upregulation of NO production by retinoids might be a sustained event for cellular survival, differentiation, and development as well as cancer progression and preventive processes in human skin cells. Thus, this study now shows that all-*trans* retinoic acid and 13-*cis* retinoic acid can regulate a cellular proliferation enzyme, *i.e.*, iNOS, by modulating its activity; hence they have promise as potential stimulators of NO synthase activity. Studies are in progress to elucidate the mechanism by which retinoids serve as potential regulators of iNOS activity, and to evaluate whether they can be useful in conjunction with chemoprevention in human skin cells (Lippman and Meyskens, 1987; Lippman et al., 1987; Lotan, 1996; Freemantle et al., 2003; Okuno et al., 2004; Moon, 2007).

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CONFLICT OF INTEREST

The author has no conflict of interest regarding the publication of this article.

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